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(57) Abstract

Methods for determining a function of cells, which comprises a suspension of cells flowing along a first fluid channel. The cells have a first detectable property associated therewith, and wherein the cells produce a second detectable property upon activation of the function of the cells, the first and second detectable properties being distinguishable from each other. The levels of the first and second detectable properties are measured. The level of second detectable property is compared to the level of first detectable property to determine the relative function of the cells.

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HIGH THROUGHPUT METHODS, SYSTEMS AND APPARATUS FOR PERFORMING CELL BASED SCREENING ASSAYS

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application is a continuation-in-part of U.S. Patent Application No.09/104,519, filed June 25, 1998, and also claims priority to Provisional U.S. Patent Application No. 60/117,370, filed January 27, 1999, each of which is incorporated herein by reference for all purposes.

BACKGROUND OF THE INVENTION

Many aspects of biological research rely upon the ability to perform extremely large numbers of chemical and biochemical assays. Increasing the throughput of screening assays has allowed researchers to adopt a more generalized approach to the overall screening process, as opposed to a more rational, predefined process. For example, in the pharmaceutical discovery process, large libraries of different compounds are generally screened against defined target systems to determine whether any of those compounds have a desired effect on that system. Once a compound is identified to have the desired effect, it is then subjected to more rigorous analysis.

Many high-throughput screening assay systems rely upon entirely in vitro models of biological systems. This is due, at least in part, to the ability to accurately control substantially all of the parameters of the model system that is being assayed to permit correlation from assay to assay, such as the quantity and purity of reagents, the environmental conditions of the assay, the operator performing the assay, and the like. Specifically, variation of any of these parameters can produce widely varying results in the performance of a given assay.

In many cases, these in vitro systems have proven to be effective models of the biochemical system of interest, and have led to the identification of promising pharmaceutical candidate compounds. However, in many instances it is desirable to use a model system that is a closer representation of what actually occurs in more complex systems, e.g., in vivo. Cell-based systems offer a closer model to these relevant biological systems, and have generally been

widely adopted as screening assays. While cell-based assays are generally preferred in screening applications, these assays have proven somewhat difficult to adapt to conventional notions of high-throughput and even ultra high-throughput screening assay systems.

As the multiplicity of cell-based assays increases, it becomes extremely advantageous to miniaturize the assay geometry. In the first instance, this miniaturization increases the efficiency of the assay by optimizing space utilization, reducing assay volumes, and consequently reduces reagent consumption and assay costs. For example, cells themselves, being a consumed reagent in such assays, are an expensive and perishable component of these assays, and quickly become a limiting influence on the application of these assays to high-throughput systems. Again, by miniaturizing assay geometries, the amount of this consumable reagent is reduced.

In addition to the economies of miniaturization, described above, a number of assay parameters become more and more critical as assay volumes are decreased. First, cells require a nutrient medium having a controlled pH in sufficient quantity to sustain their continued viability. Second, the cells need to be protected from desiccation, which is a particular problem in very small fluid volumes. Third, otherwise simple manipulations, such as reagent addition, rapid mixing and sampling become very difficult when dealing with extremely small fluid volumes. Further, continuous and accurate kinetic reading of assay results, e.g., monitoring of signals, during and after reagent or sample addition is a necessary element of many cell-based assays. Often these assay results come in the form of very small changes in signal levels from the cells, e.g., intracellular or membrane associated fluorescent signals. These small changes become increasingly difficult to detect as assay volumes are decreased and signal to noise ratios decrease.

Accordingly, it would generally be desirable to provide methods, devices and systems for performing cell based assays that are readily adaptable to high throughput screening applications, are readily automated, are easily repeated, and require less reagents and/or other assay components. The present invention meets these and a variety of other needs.

SUMMARY OF THE INVENTION

In a first aspect, the present invention provides methods of determining a function of cells, which comprises a suspension of cells flowing along a first fluid channel. The cells have a first detectable property associated therewith, and wherein the cells produce a second

detectable property upon activation of the function of the cells, the first and second detectable properties being distinguishable from each other. The levels of the first and second detectable properties are measured. The level of second detectable property is compared to the level of first detectable property to determine the relative function of the cells.

The present invention also provides an apparatus for measuring a function of cells, comprising a body structure having a first fluid channel disposed therein. The first fluid channel is in fluid communication with a first source of a suspension of cells and the cells have a first detectable property associated therewith. The cells produce a second detectable property upon activation of the function of the cells, the first and second detectable properties being distinguishable from each other. The apparatus also optionally includes a material transport system for flowing the suspension of cells along the first channel and a detector for detecting and distinguishing the first detectable property from the second detectable property associated with cells within the first channel.

The present invention also provides methods of measuring a binding function of a cell, comprising a channel disposed in a first body structure. The channel comprises a first binding region and a non-binding region, the first binding region having a binding moiety immobilized on an interior surface of the first channel therein. A suspension of cells flows along a first channel, the cells comprising on their surfaces, a moiety specifically bound by the binding moiety. A relative velocity of cells flowing through the binding region is determined, relative to a velocity of cells flowing through a non-binding region. A decrease in the relative velocity is indicative of first binding in the binding region.

The present invention also provides an apparatus for measuring a binding function of a cell, using a body structure comprising a first channel disposed therein. The channel includes a binding region, a non-binding region, a binding moiety immobilized on an interior surface of the first channel in the binding region but not the non-binding region, a source of a suspension of cells in fluid communication with the first channel, a means for flowing the suspension of cells along the first channel, and a detection system for determining a relative velocity of cells flowing through the binding region compared to a velocity of cells flowing through the non-binding region.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a simplified illustration the detected signal profiles obtained using the methods and systems of the present invention. Figure 1A illustrates the level of reference signal and function signal from a particular cell suspension over time, e.g., as it flows past a detection point. Figure 1B illustrates a comparison plot of detected function label versus reference label, and an approximate slope calculation for that comparison. Figure 1C illustrates the level of reference and function label in the same system, but where the assayed function is reduced over the system shown in Figure 1A, e.g., in the presence of an inhibitor or absence of an enhancer. Figure 1D illustrates a comparison plot of the function and reference signals from Figure 1C, and an approximate slope calculation for that comparison.

Figure 2 is a schematic illustration of an overall system for carrying out the assay and screening methods of the invention.

Figure 3 is a schematic illustration of a microfluidic device incorporating a layered body structure.

Figure 4 is a simplified schematic illustration of a microfluidic device for screening multiple test compounds for effects on cells in accordance with the assay methods of the present invention.

Figure 5 is a schematic illustration of an integrated system for accessing and screening large numbers of test compounds against cellular systems in accordance with the methods of the present invention.

Figure 6 is a schematic illustration of an optical detection system for separately detecting reference and function labels from cell suspensions.

Figures 7A-7E show a number of scatter plots of CHO cells stained with Syto-17 (a nucleic acid stain) and with an intracellular calcium indicator (Fluo-3), both in the absence (7A) and presence of varying levels of ionomycin (7B-7E), a known ionophore for calcium that causes increases in intracellular calcium levels. Figure 7F shows a dose response curve for ionomycin in CHO cells.

Figure 8 is a diagram of a microfluidic device used in demonstrating the assay methods of the present invention.

Figure 9 is a scatter diagram of THP-1 cells bearing a reference label (Syto-17-nucleic acid dye) and a function label (Fluo-3- intracellular calcium indicator). The lower group of plotted points correspond to the control cells while the upper group of plotted points

corresponds to cells in the presence of UTP, a known agonist of P2Y receptors that are coupled to intracellular release of calcium stores by the Gq signal transduction pathway.

Figure 10 is a plot of the dose response of intracellular calcium flux in THP-1 cells to varying levels of UTP.

Figure 11 is a plot of the kinetics of the stimulation of calcium transport with UTP. In particular, intracellular calcium levels are measured as a function of time from contact with UTP, by varying the distance between the detector and the point at which UTP was introduced into the analysis channel.

Figure 12 is a graph of Syto 62 stained cells indicating total cell count (bottom line) and Fluorescein FragEL labeled cells (top line) for cells not treated with Campthotecin.

Figure 13 is a graph of Syto 62 stained cells indicating total cell count (bottom line) and Fluorescein FragEL labeled cells (top line) for cells treated with Campthotecin.

Figure 14 is a graph of cells stained with Calcein (bottom line) and cells labeled with Annexin-V-Cy5 (top line). Cells labeled with both dyes are apoptotic, cells labeled with Calcein only are live and not apoptotic, and cells labeled with Annexin-Cy5 only were dead.

Figure 15 is a graph of cells stained with Calcein (bottom line) and cells labeled with Annexin-V-Cy5 (top line) after treatment with Campthotecin. Cells labeled with both dyes are apoptotic, cells labeled with Calcein only are live and not apoptotic, and cells labeled with Annexin-Cy5 only were dead.

DETAILED DESCRIPTION OF THE INVENTION

I. Assay Methods

In general terms, the present invention provides methods, systems and apparatus for assaying biological functions. These methods, systems and apparatus are typically employed in assaying cells for a particular biological function, and in particularly preferred aspects, for screening test compounds for their effects, if any, on the biological function of cells. As used herein, the phrase "a function of cells" or "cellular function" generally refers to a selected biological activity or biological activities of cells. These functions include, without limitation, the full range of anabolic and catabolic reactions that occur within or at the surface of cells. Functions of cells range from specific predefined biochemical interactions, i.e., receptor/ligand binding, to the more general reactions and/or interactions, i.e., initiation of signaling cascades

and overall cell viability. It will be readily appreciated that the cellular functions that may be assayed in accordance with the present invention are generally limited only by one's ability to detect that function or the results of that function. Preferred assayable functions include those that are generally considered to be pharmacologically relevant, are linked to a particular disease, disorder, or the like. Some general examples of these cellular functions include transport functions, i.e., ion channel activation, binding functions, i.e., ligand/receptor binding, nucleic acid hybridization, expression functions, i.e., gene expression and protein translocation, and overall cellular viability.

The present invention generally provides methods of assaying these cellular functions by flowing a suspension of cells along a first channel where the cells have a first detectable property associated with them. The first detectable property generally comprises a characteristic that is substantially uniformly associated with all of the cells that are being assayed. As such, this first detectable property functions as a "reference" label, to indicate the relative presence of cells within the assay system, e.g., that are being detected at any given time. As used herein, the phrase "substantially uniformly associated with cells in the suspension," means that the reference label will be present in/on all of the cells, or a selected subset of cells in a suspension at approximately the same level, e.g., with less than 20% variation, preferably less than 10 % variation, and more preferably, less than 5% variation from one cell to another. The uniformity of the first detectable property among all cells allows for the relative quantitation of cells or subset of cells that are being interrogated at any given time in the assay, e.g., those that are within the field of detection.

By measuring the relative presence of cells within the measurement or detection region, the methods and apparatuses described herein provide measurements of cell function that are self-corrected for cell number. Thus, the methods of the present invention typically measure the reference and function labels from a plurality of cells, e.g., 2, 10, 100 or more, simultaneously.

The methods of the present invention are useful in measuring functions of virtually any type of cells, including, mammalian, bacterial, fungal, yeast, insect, and plant cells. In particularly preferred aspects, mammalian cells, e.g., CHO, THP-1 cells, blood cells, i.e., B cells, T cells , monocytes and neutrophils, and bacterial cells are used to screen for agents that affect these cell types, e.g., pharmaceutical agents, antibiotic agents, and the like.

A simplified example of the signal profile from both the reference and function signals and their comparison, is shown in Figure 1. In particular, Figure 1A illustrates the signal output from a scanner that detects and separately quantifies the reference signal (solid line) and the function signal (dotted line), e.g., a dual wavelength fluorescent detector. The signals are produced as a suspension of cells bearing the reference and function labels are flowed through a channel past the detector. The different peaks (denoted by the circle, triangle and square) represent different cells or groups of cells that pass the detector over time. The larger reference peaks correspond to larger groups of cells that are being detected at the particular time. Figure 1B illustrates a comparison plot of the reference signal versus the function signal, where the slope (approximately 2.0) of the line is an indication of the relative function of each cell in the suspension.

Figure 1C illustrates a similar plot of both reference and function signals as Figure 1A, but in the presence of an inhibitor. As shown, the size of the function signal for each peak is reduced relative to the size of their corresponding reference peaks. This size differential is quantified in the comparison plot in Figure 1D. In the presence of the inhibitor, the slope is reduced to 1.5, giving a quantitative indication of the amount of inhibition in the assay shown in Figure 1C over that shown in Figure 1A.

A variety of different detectable properties may be used as the reference label, in accordance with the present invention. For example, in some cases, inherent or native properties or characteristics of the cells are optionally employed as the first detectable property or reference label. While such inherent or native properties or characteristics, e.g., light scattering, flow characteristics, etc., may be used in some cases as the reference label, in preferred aspects, light scattering or other native characteristics are not used as the first detectable property, as the level and specificity of detectable signal from these characteristics is extremely low on a per cell basis. Instead, non-native labeling schemes are preferred for use in accordance with the present invention, for their ability to produce much higher and more specific signals. By "non-native" labeling scheme is meant the incorporation of a detectable property within a cell or cell line, that is not naturally associated with the cell or cell line. Non-native reference labels include: associative labels, e.g., labeling groups that are added to the cells and which associate with a portion of the cells of interest; expressed labels, e.g., labeling groups that are constitutively expressed from a recombinant gene construct that is incorporated into the cells; and generated labels, e.g., labels that are produced as a result of some constitutive activity of the cells, e.g.,

energy utilization, generation of by-products, etc. In order to maximize sensitivity, reference labels are preferably selected from chromophoric labels (chromophores), chemiluminescent labels, fluorescent labels (fluorophores) or electrochemical labels, with energy emitting reference labels, e.g., fluorescent or chemiluminescent labels, being most preferred.

In certain aspects, associative labels are used as the first detectable property or reference label. In order to avoid interfering effects from the reference labels, it is generally desirable to select reference labels that do not have any effect on the cellular function that is to be assayed. In particular, associative reference labels are generally selected so as to avoid any activating influence on the cells, or any interaction with cell elements involved in the assayed function.

Examples of preferred reference labels include nucleic acid associating fluorescent labels. These labels generally associate with the nucleic acids that are present in the cells, and are therefore generally uniformly incorporated into all of the cells in the suspension. Commercially available examples of nucleic acid labels include, e.g., the SYTO series of dyes available from Molecular Probes, Inc., e.g., SYTO-17, which excite in the visible range of the spectrum, and may be selected depending upon a number of characteristics, including cell permeability, fluorescence enhancement upon binding nucleic acids, excitation and emission spectra, DNA/RNA selectivity and binding affinity. Protocols for incorporating these labels in the cells that are to be assayed are generally well known in the art, and/or are available from the manufacturer of the labels.

Alternatively, labels that associate with cell membranes, e.g., lipophilic labeling groups, or that associate with cell membrane components, e.g., cell surface proteins, in a passive or non-activating manner, are used to uniformly label all of the cells that are being assayed. A variety of membrane associative labels are commercially available and include, e.g., lipophilic fluoresceins such as acylaminofluorescein (tetrabromofluorescein, 5-dodecanoylaminofluorescein, 5-hexadecanoylaminofluorescein and 5-octadecanoylaminofluorescein), lipophilic rhodamines (octadecyl rhodamine B), alkylated coumarins, acridines and resorufin, and the like. These labeling materials are generally commercially available from, e.g., Molecular Probes, Inc. Again, labeling protocols are generally available from the manufacturers of these labels, and are well known to those of skill in the art.

Because the methods of the invention are self-correcting for cell concentrations, a wide range of cell concentrations are optionally used. However, in preferred aspects, the cell suspension is generally provided at a cell concentration that maximizes the sensitivity of the detection process, while minimizing negative effects of excessive cell concentrations, e.g., negative flow properties (i.e., clogging, excessive viscosity, excessive aggregation) excessive accumulation of deleterious by-products etc., that might effect assay results, and the like. As such, the cell suspensions are generally provided at cell concentrations between about 1×10^5 to about 1×10^7 per ml. Cell concentrations generally vary within this range, depending upon the nature of the channel through which the cells are being flowed during the assay process. For example, for narrower channels, more dilute cell suspensions are generally used, while for larger channels, higher cell concentrations can be tolerated. Of course for different cell types and sizes, concentrations outside this range are also envisioned. For example, for larger cell types, e.g., VB-2 cells, more dilute cell suspensions are used, while for smaller cell types, e.g., PBLs, more concentrated suspensions are used. The "suspensions of cells" discussed herein also encompasses cells that are adhered or immobilized to suspendable solid supports, e.g., beads (carbohydrate beads, latex microspheres, controlled porosity glass beads, and the like). Suspensions of beads carrying adhered cells are used in the same fashion as pure cell suspensions, as described herein.

Although described in terms of labels that are ubiquitously associated with the cells in the suspension, it should be understood that the reference label may be specific to a particular or distinct subset of cells within a given suspension, such that a function of that subset may be quantified in the overall suspension. As used herein, the phrase "distinct subset of cells" means a group of cells within a larger population of cells that has distinct functional, morphological, or genotypical characteristics, such that these cells can be separately identified and characterized from the remainder of the cell population. For example, in screening blood samples, reference labels may be selected that are specific to either white cells or red cells, or subsets thereof, e.g., labeled antibodies that are specific for B cells, T cells, monocytes, neutrophils, and the like. Function labels are then selected to indicate the level of a function of the particular cell subset.

In accordance with the present invention, the cells that are being assayed also include a second detectable property that results from the particular cellular function that is being assayed, also termed a function label. Function labels are usually selected depending upon the

particular function that is being assayed. Types of assays and their function labels are generally described in greater detail, below.

As with the reference label, the function label may be an inherent or native characteristic that naturally results from the function of the cells, such as changes in the media composition, e.g., pH variations, and the like. Again, however, non-native labels and particularly energy emitting labels are preferred for use in accordance with the present invention. A variety of function labels are well known in the art, and are generally described in greater detail below.

Function labels are generally selected and/or provided such that they are readily distinguishable from the first detectable property, or reference label. The term "distinguishable" when used to describe the reference and function labels of the present invention, denotes two detectable properties that can be separately detected, and their levels separately quantified, using single or multiple detection systems. For example, the reference label may be detectable using an electrochemical label and detection system, while the function label is detectable using a fluorescent label and detection system. As a result, detection of the reference label does not substantially overlap or interfere with the detection of the function label. More typically, both the reference label and function label comprise fluorescent labeling groups. Again, however, the fluorescent reference and function labels are selected to be distinguishable from each other.

Fluorescent labeling groups are generally distinguishable from each other based upon one or more of their excitation spectra, emission spectra or fluorescent lifetimes. Specifically, the reference label may have an excitation maximum, e.g., wavelength of activation light required to cause the fluorescent groups to fluoresce, that is substantially different than the excitation maxima of the function label. By separately directing excitation light of different wavelengths at the cells, one could then determine the level of fluorescence resulting from the reference label versus the function label. Alternatively, the reference label and function label are selected to have distinguishable fluorescent emission maxima, e.g., they emit light or fluoresce at substantially different wavelengths. In operation, a single light source is directed at the cells. The fluorescent emissions from the cells are then passed through optical filters, which separate the different fluorescent emissions, which are then separately quantified. In selecting either distinguishable excitation or emission maxima, it is generally preferred that the excitation or emission spectrum of one label, e.g., the reference label, does not appreciably overlap with the excitation or emission spectrum of the other label. Specifically, while there is generally a

maximum excitation or emission wavelength for different labels, there is typically a broader range of wavelengths at which there is some excitation or emission. Typically, labels are selected such that there is substantially no overlap between the excitation or emission spectra of the two labels, e.g., in detection of one label, less than 10% of the fluorescence is due to overlap from the other label.

Multi-wavelength detection schemes and systems have been described for use in a large number of different analytical systems, including macromolecular separations, e.g., sequencing of nucleic acids and the like (See U.S. Patent No.5,171,534), nucleic acid array scanning, and the like.

The first and second detectable properties, or the reference label and function label, are then measured in the flowing cell suspension. By comparing the level of function label to the level of reference label, one can identify and quantify any increases or decreases in the function of the cells, regardless of the number of cells that are detected. Specifically, by comparing the level of function label to the level of reference label, one can readily determine if an increase in the level of function label is a result of increased cellular function, or simply an increase in the number of cells that are detected. Specifically, the reference label provides an indicator of the number of cells that are subjected to measurement at a given time during the assay, while the function label, provides an indication of the amount of cellular function resulting from that number of cells.

The ability to quantitatively determine cellular function within a single channel using the presently described methods, provides significant advantages over previous cell-based assay systems, e.g., flow cytometry, where optimal assay results are obtained from individual separated cells, rather from larger numbers of cells. Because separation of individual cells is not necessary to practice the methods described herein, these methods also require much less sophisticated equipment, e.g., fluid handling, detection systems, and the like. Data acquisition from individual cells further suffers from problems of cell to cell variations, and the like. In particular, in flow cytometry methods, large numbers of individual cells must be individually assayed in order to observe data trends, whereas the methods of the present invention observe and detect such trends much more quickly, e.g., with each data point.

Other cell based assay systems, such as flow cytometry, also suffer from an inability to easily add affector agents and kinetically monitor the cell response. The methods and apparatuses of the present invention, on the other hand are readily employed in adding affector

agents and monitoring their affects, kinetically, on the cells in the assay. Specifically, incubation times of cells and affector agents are generally altered by simply altering the amount of incubation time prior to detection. In the methods and apparatuses described herein, this is simply done by either altering the length of the incubation channel, varying the point along the detection channel at which the assay results are detected, or slowing the flow rate along the reaction channel. Kinetics are also easily monitored by incorporating additional detectors, e.g., one or more additional detectors, at different points along the reaction channel.

Other cell-based assays, e.g., those carried out in multi-well plates, also suffer from substantial variations due to variations in the number of cells present within the reading area of the detection system. In particular, this can occur when affector agents are rapidly added, which can dislodge cells from the surfaces of the wells, causing those cells to move into and out of the reading area.

As noted above, the assay methods of the present invention are typically used in screening test compounds for their ability to affect cellular functions. In performing these screening assays, the cells that are to be assayed are exposed to different test compounds or conditions. In screening libraries of compounds, these compounds are typically separately introduced into the flowing suspension of cells. The relative level of a particular cellular function is then compared to a control system, e.g., the cells in the absence of the compound or condition, to determine whether the compound or condition has an effect on the cellular function. For example, where the level of relative cellular function decreases in the presence of a compound, it will be presumed that the compound possesses an inhibitory activity toward the cellular function. Conversely, where a cellular function is increased in the presence of the compound, it is assumed that the compound provides an enhancing activity to the cellular function. In either event, compounds that show some effect on cellular function are then subjected to more pointed analyses to elucidate their precise activity with respect to the cellular function.

II. Assay Types

A. Assaying Relative Cellular Function

As noted above, the methods and systems of the present invention are useful in assaying for virtually any cellular function, provided that either the function or a result of the function is independently detectable. In biological applications, and particularly pharmaceutical

research, a number of specific types of assays are generally used as screening models for the identification of potential drug candidates, or "lead compounds." The assay types most frequently used in these screening operations generally include transport assays, binding assays, viability assays and expression assays.

1. Transport

In a first aspect, the methods and systems of the present invention are used in assaying cellular transport functions, i.e., ion flux, and intracellular pH regulation. In particular, cellular transport channels have been generally shown to be responsive to important cellular events, e.g., receptor mediated cell activation, and the like. For example, G-protein coupled receptors have been shown to directly or indirectly activate or inactivate ion channels in the plasma membrane or endosomal membranes of cells, thereby altering their ion permeability and thus effecting the excitability of the membrane and intracellular ion concentrations. See, Hille, Ionic Channels of Excitable Membranes, Sinauer Assoc. (1984).

In accordance with this aspect of the present invention, therefore, the function specific label comprises an indicator of the level of a particular intracellular species. In particularly preferred aspects, the intracellular species is an ionic species, such as Ca^{++}, Na^{+}, K^{+}, Cl^{-}, or H^{+} (e.g., for pH measurements). A variety of intracellular indicator compounds are commercially available for these ionic species (e.g., from Molecular Probes, Eugene OR). For example, commonly used calcium indicators include analogs of BAPTA (1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid), such as Fura-2, Fluo-2 and Indo-1, which produce shifts in the fluorescent excitation or emission maxima upon binding calcium, and Fluo-3 and Calcium Green-2, which produce increases in fluorescence intensity upon binding calcium. See also, U.S. Patent No. 5,516,911. Sodium and potassium sensitive dyes include SBFI and PBFI, respectively (also commercially available from Molecular Probes). Examples of commercially available chloride sensitive indicators include 6-methoxy-N-(sulfopropyl)quinolinium (SPQ), N-(sulfopropyl)acridinium (SPA), N-(6-methoxyquinolyl)acetic acid, and N-(6-methoxyquinolyl)acetoethyl ester (Molecular Probes, Inc.), all of which are generally quenched in the presence of chloride ions.

In a related aspect, the function specific indicator is an intracellular pH indicator compound. Specifically, intracellular pH changes have been found to be associated with biologically and pharmaceutically important cellular events, including cell proliferation, apoptosis, fertilization, malignancy, ion transport, drug resistance, lysosomal storage disorders,

and Alzheimer's disease. A variety of indicator compounds are commercially available to indicate the intracellular pH of cells, and are readily applicable to the present invention as indicators of cellular function. Examples of these pH indicators include, e.g., SNARFL, SNARF, BCECF, and HPTS, available from Molecular Probes, Inc.

In operation, a suspension of cells that is to be assayed is flowed along a channel. The cells include a reference label as described above, i.e., SYTO dyes available from Molecular Probes. The cells are also treated with an intracellular indicator of the level of the species for which relative transport levels are to be determined, and which indicator is distinguishable from the reference label. As a specific example, the cells are optionally stained with, e.g., SYTO-17 as a reference label. SYTO-17 is a red nucleic acid dye that is generally excited by light at approximately 621 nm, and which emits light at approximately 634 nm. The cells are also optionally treated with an intracellular calcium indicator, e.g., Fluo-3, also available from Molecular Probes, which is excited at 488 nm and emits at approximately 530 nm. The two labels are easily distinguishable based upon their differing fluorescent emission maxima.

At a point in the channel, the cells are illuminated with a broad spectrum of light, e.g., light that encompasses the excitation maxima of both the SYTO-17 and Fluo-3 labels. Emitted fluorescence is then passed through optical filtering systems that separate and separately detect the SYTO-17 fluorescence and the Fluo-3 fluorescence. The levels of fluorescence from each dye are then compared (see e.g., Fig. 6). For example, the comparison optionally includes plotting the level of reference label versus the level of function label. Over the course of the assay, a number of separate data points are gathered that represent different cells or groups of cells that are detected. These are plotted and the slope of the resulting line is calculated. Changes in this slope are indicative of changes in the level of the function that is being assayed (see, e.g., Fig 7A-7E and Fig. 9).

2. Binding

a) Generally

In an alternate aspect, the methods and systems of the present invention are used in assaying cellular binding functions, such as ligand-receptor binding, nucleic acid hybridization, antigen/antibody binding, cell-cell interactions, and the like. As with transport functions, cellular binding functions are often necessary precursors to a variety of cellular functions. Specifically, many biological responses are often triggered and/or controlled by the binding of a receptor to its ligand. For example, interaction of growth factors, i.e., EGF, FGF,

PDGF, etc., with their receptors stimulates a wide variety of biological responses including, e.g., cell proliferation and differentiation, activation of mediating enzymes, stimulation of messenger turnover, alterations in ion fluxes, activation of enzymes, changes in cell shape and the alteration in genetic expression levels. Accordingly, control of the interaction of the receptor and its ligand may offer control of the biological responses caused by that interaction. As noted, included within these biological functions controlled by binding reactions are many transport functions, e.g., G-protein linked receptor activation, as set forth above. Accordingly, these binding functions may be detected by detecting the downstream event for which binding is a precursor, e.g., enhanced or decreased transport function, expression of receptor linked reporter label, protein translocation, or by detecting actual binding of cells with a binding agent, e.g., a ligand, nucleic acid or the like, through the inclusion in the ligand of a binding indicator, e.g., fluorescent resonance energy transfer dyes (FRET), molecular beacons, etc. For example, in the case of cell-cell interactions, detection of binding may be accomplished by labeling the cells' surfaces with both elements of appropriate FRET dyes, e.g., energy donor and energy acceptor. Upon cell-cell binding, these elements are placed in sufficient proximity for energy transfer, allowing their detection.

Alternatively, fluorescence polarization detection methods are used to detect binding of relatively small molecules, e.g., ligands, antibodies, etc., to relatively large structures, e.g., cells. Fluorescence polarization assays for use in microfluidic systems are generally described in Provisional U.S. Application No. 60/088,650, filed June 8, 1998, incorporated herein by reference.

A variety of other detection/labeling mechanisms are also available for detecting binding of one molecule, e.g., a ligand or antibody, to another molecule, e.g., a cell surface receptor. For example, a number of labeling materials change their fluorescent properties upon binding to hydrophobic sites on proteins, e.g., cell surface proteins. Such labels include, e.g., 8-amino-1-naphthalene sulfonate (ANS), 2-p-toluidinylnaphthalene-6-sulfonate (TNS) and the like. Alternatively, detectable enzyme labels are utilized that cause precipitation of fluorescent products on solid phases, i.e., cell surfaces are optionally used as function indicators of binding. For example, alkaline phosphatase substrates that yield fluorescent precipitates are optionally employed in conjunction with alkaline phosphatase conjugates of cell binding components. Such substrates are generally available from Molecular Probes, Inc., and are described in, e.g., U.S. 5,316,906, U.S. 5,443,986.

b) Cell Rolling Assays

In a related but alternative aspect, the present invention provides methods, devices and systems for use in performing in vitro cell rolling assays. In particular, it has been reported that several classes of cell adhesion molecules participate in a wide range of important physiological functions, including wound healing, inflammation and cancer metastasis. Some examples of these molecules include selectins and integrins which mediate the rolling and subsequent immobilization of white blood cells along the endothelial lining of blood vessel, thus allowing then to migrate out of the blood vessel and toward the target tissue. Cell rolling assays are designed to mimic in vitro the rolling phenomenon in vivo, to create a more effective model for use in screening potential effectors of that phenomenon. Lawrence et al., J. Immunol., (1993) 151:6338-6346; Brunk et al., Biophys. J. (1997) 72:2820-2833.

Generally, the assay is performed by flowing a suspension of cells over a surface upon which ligands are immobilized, and observing the numbers of firmly attached and/or rolling cells on that surface as well as the velocity of the rolling cells. The present invention employs the microfluidic systems described herein, in the performance of these assay types. In particular, as described in greater detail below, the cell suspension bearing an appropriate reference label, is introduced into a channel in which an appropriate ligand of interest is immobilized on the inner surface. Immobilization of ligands on the interior surface of channels is optionally accomplished by covalently attaching the ligands to the surface or by adsorbing the ligands on the surface. Covalent attachment of ligands to surfaces of solid substrates as been described in the art. See, e.g., Sundberg, et al., J. Am. Chem. Soc. (1995) 117:12050-57.

In accordance with the present invention, the cell suspension is flowed through the channel, i.e., using pressure flow as described in greater detail below, and the number of cells that are rolling over or firmly attached to the interior surface of the channel is monitored using an appropriate detection system. Alternatively, cells are pulsed through the channel to facilitate their monitoring. Typically, such systems employ a video imaging system that images and identifies the cells as they enter the imaged area, and tracks the cells path through the field, determining their relative velocity. Alternatively, point detection systems, e.g., as described herein, are used which detect cells at two separate points in the channel, and determine their relative velocity. In the latter case, it is generally desirable to provide the cells in suspension that is sufficiently dilute so as to permit correlation between the two detectors. Alternatively, cells may be coded with mixtures of different, distinguishable labels to permit the correlation among

cells between points. Such coded cells may include wide varieties of different labels, or alternatively, may include a set of two, three, four, five, six, seven or eight different labels at varying relative levels, where the profile of the relative levels of labels identifies the different cells.

In screening assays, the test compounds are introduced into the analysis channel, e.g., via an external sample accessing capillary (i.e., an electrokinetic injector) where they contact the suspension of cells. The cell suspension is then assayed for rolling or firmly attached cells, and the effect of the test compound, if any, on the cell rolling or binding is determined as compared to the control, e.g., in the absence of the test compound.

3. Viability

In still another respect, the methods and systems of the present invention are also particularly applicable in performing cell viability assays, and particularly for screening test compounds for their effects on cell viability. Such assays are generally utilized in performing toxicity studies, antibiotic screening methods, and the like, and are particularly suitable for the methods and systems of the present invention. Accordingly, in these aspects, the cellular function specific indicator is an indicator of cell viability.

In operation, the suspension of cells includes a reference label as described above. The cells are also treated with a second function labeling group that indicates the viability of the cells in the suspension. Specifically, the function label preferentially stains or labels either viable or non-viable cells. A variety of viability indicative dyes are generally commercially available. For example, fluorogenic esterase substrates, such as calcein AM, BCECF AM and fluorescein diacetate, can be loaded into adherent or nonadherent cells, and are suitable indicators of cell viability. Specifically, these esterase substrates measure both esterase activity, which is required to activate the fluorescence of the dye, as well as cell-membrane integrity, which retains the fluorescent materials intracellularly. Other suitable viability indicators include polyfluorinated fluorescein derivatives (i.e., DFFDA, TFFDA, HFFDA and Br_4TFFDA), polar nucleic acid based dyes (i.e., SYTOX Green™), dimeric and monomeric cyanine dyes (i.e., TOTO™ and TO-PRO™ series dyes from Molecular Probes), ethidium and propidium dyes (i.e., ethidium bromide, ethidium homodimer and propidium iodide).

Depending upon the viability indicator used, the level of function label is indicative of the number of either viable or non-viable cells, while the level of reference label is indicative of the number of total cells, e.g., viable and non-viable. Comparison of the levels of

the two labels then provides an indication of the relative viability of the cells in the suspension, regardless of the number of cells being detected, e.g., multiple cells, aggregates, or individual cells). In particular, where two cell populations show a similar level of reference label, but one population shows a lower level of viability indicator, it will be understood that the second population is less viable, e.g., has more nonviable cells. It will be appreciated that many dyes or labels described for use as reference labels are also often used as viability labels. Accordingly, it will generally be desirable to select a reference label that labels both viable and nonviable cellular material, and which is distinguishable from the function label. Examples of such reference labels include, e.g., lipophilic membrane labels, and the like.

In performing screening assays, cell suspensions that are exposed to different test compounds or agents are flowed past the detection point and the relative viability of the cells is determined, as compared to a control. Increases or decreases in cellular viability indicate that the compound or agent improves or decreases cellular viability. Such assays are readily employed in identifying antimicrobial, antibiotic or other viability affecting agents. Similarly, such assays are optionally employed in screening for effectors of pathways involved in apoptosis or programmed cell death, e.g., caspase mediated (growth promoting) pathways.

4. Expression

In a further aspect, the methods and systems of the present invention are used to assay cellular expression functions, and particularly, for the effect of test compounds on such cellular expression. Such assays are generally utilized in screening for effectors of given biological processes, which effectors target those functions at the gene expression level. In accordance with the present invention, therefore, the function label is indicative of the level of gene expression, for a particular gene of interest.

Gene expression levels are typically assayed by quantifying the level of gene product from the gene of interest, e.g., the amount of protein produced by the cells. Alternate methods of gene expression analysis examine the amount of RNA transcribed from the gene of interest. Typically, such assays involve the use of a nucleic acid hybridization assay to identify a pattern of RNA transcription following an activating event.

The methods and systems of the present invention are readily applied to such expression analyses. In particular, in accordance with the present invention, the function label is typically provided that is expressed by the cells during the expression function. For example, chimeric reporter systems may be employed as function labels or indicators of gene expression.

Chimeric reporter systems typically incorporate a heterogeneous reporter system integrated into the coding sequence of the gene of interest. The expression of the gene of interest is then accompanied by the expression of the reporter, which is then detected. For example, a receptor may be fusion between the product of the gene of interest and heterologous protein, e.g., an enzyme whose activity is readily assayable, or an otherwise detectable protein, e.g., luciferase, aequorin, green fluorescent protein (GFP), β-galactosidase, alkaline phosphatase, or the like. The expressed reporter is then detected and compared with the level of reference label, to provide a quantitative determination of expression levels on a per cell basis. Expression of gene products to a detectable level can require varying amounts of time, e.g., several minutes to hours. Accordingly, the assay time is varied to allow such expression. As noted herein, such variation is generally accomplished by one or more of slowing the flow rates of the cell suspension through the analysis channel and/or lengthening the analysis channel.

Alternatively, the function label is provided as an element of a binding molecule that specifically associates with the downstream indicator of gene expression, e.g., an expressed protein, wherein the binding of the binding molecule (bearing the function label) to the gene product of interest produces a detectable property within the cell, e.g., as described with reference to the binding assays, above. The assay methods are then carried out in the same manner as described with reference to the binding functions, described above. In the case of expressed proteins, the binding molecule optionally includes an antibody specific for the gene product, or a specific binding partner, where the expressed protein is a member of a binding pair, e.g., a receptor or ligand.

Because gene expression assays typically require much longer incubation times than other assay types described herein, modified methods are optionally employed. For example, in one aspect, cells are flowed through the channel of the system, and preferably, through multiple parallel channels of the system, and contacted with surfaces of the channel(s) that cause them to adhere. Test compounds are then introduced into the channel(s) and allowed to flow over the adhered cells, e.g., for from 5 to 60 minutes. Any effects of these test compounds on the level of function label, and therefore, gene expression, is determined in the channel(s), and compared to the level of reference label. The comparison then allows quantification of the level of expression on a per cell basis. Optionally, the reaction channel is provided such that the travel time of the cells from the point of test compound addition to detection is sufficient to permit appropriate expression analysis. In certain aspects, tortuous

channels, e.g., serpentine channels, and the like, are used to extend channel lengths to expand the travel time. Alternatively or additionally, flow rates are substantially reduced to increase this travel time.

B. Screening Assays

As noted repeatedly above, the assays described herein are particularly useful in performing high-throughput screening assays. As used herein, the term "test compound" refers to the collection of compounds that are to be screened for their ability to affect a particular biochemical system. Test compounds may include a wide variety of different compounds, including chemical compounds, mixtures of chemical compounds, e.g., polysaccharides, small organic or inorganic molecules, biological macromolecules, e.g., peptides, proteins, nucleic acids, or an extract made from biological materials such as bacteria, plants, fungi, or animal cells or tissues, naturally occurring or synthetic compositions. Depending upon the particular embodiment being practiced, the test compounds may be provided, e.g., injected, free in solution, or may be attached to a carrier, or a solid support, e.g., beads. A number of suitable solid supports may be employed for immobilization of the test compounds. Examples of suitable solid supports include agarose, cellulose, dextran (commercially available as, i.e., Sephadex, Sepharose) carboxymethyl cellulose, polystyrene, polyethylene glycol (PEG), filter paper, nitrocellulose, ion exchange resins, plastic films, glass beads, polyaminemethylvinylether maleic acid copolymer, amino acid copolymer, ethylene-maleic acid copolymer, nylon, silk, etc. Additionally, for the methods and apparatuses described herein, test compounds may be screened individually, or in groups. Group screening is particularly useful where hit rates for effective test compounds are expected to be low such that one would not expect more than one positive result for a given group. Alternatively, such group screening may be used where the effects of different test compounds may be differentially detected in a single system, e.g., through electrophoretic separation of the effects, or differential labeling which enables separate detection.

Typically, vast libraries of test compounds are separately tested for potential effects on different cellular functions In preferred aspects, large libraries of chemical compounds prepared using combinatorial synthesis techniques are typically employed as test compounds in high-throughput screening applications, to identify any such compounds that may have pharmacologically beneficial activities. In optional preferred aspects, test compounds can include large libraries of naturally occurring materials or compounds, libraries of genetic material, protein fragments, and the like.

In general, the test compounds are separately introduced into the assay systems described herein. The relative level of a particular cellular function is then assessed in the presence of the test compound, and this relative level of function is then compared to a control system, which lacks an introduced test compound. Increases or decreases in relative cellular function are indicative that the test compound is an enhancer or an inhibitor of the particular cellular function, respectively.

III. Assay Systems

A. Overall Systems

As noted above, the present invention also provides systems and devices used to practice the above-described methods. A schematic illustration of a system for carrying out these assay methods is illustrated in Figure 2. As shown, the system includes a fluidic channel 200 along which is flowed a suspension of cells 210 that bear a reference label and a function label. A source of different test compounds 220 is optionally linked to the channel 200, for introducing the different test compounds into the channel whereupon they are contacted with the cells. One or more detectors 230 are also provided in sensory communication with the channel 200, for detecting and quantifying both the level of reference label and the level of function label present on the cells. As used herein, the phrase "sensory communication" refers to orientation of the detector such that it is capable of obtaining an appropriate signal from the point of interest. In the case of optical detectors, sensory communication provides a detector oriented such that it is capable of receiving an optical signal from a channel of a microfluidic device. Such detection may be direct, or may include an intervening optical pathway, e.g., lenses, fiber optics, etc. In the case of chemical detectors, such sensory communication typically requires a sensor component disposed in contact with the fluid material within the channel.

The detector(s) is operably linked to a processor 240, e.g., a computer, for recording the detected levels of reference and function labels, and for comparing the level of function label to the level of reference label, and providing a report of relative activity of the cells that are being assayed 250. The computer 240 also typically includes appropriate programming for determining whether one assay, e.g., a first screening assay, shows greater or lesser cellular function than another assay, e.g., a control.

Detector 230 optionally includes one or more different detectors, and is selected to detect both the reference and function labels present in the cells. For example, in the case of

cells that include reference and function labels that are fluorescent, the detector typically includes a dual wavelength fluorescent detector. A schematic illustration of such a detector is shown in Figure 6. As shown, the detector 230 includes a light source 602. Appropriate light sources may vary depending upon the type of detection being employed. For example, in some cases broad spectrum illumination is desirable while in other cases, a more narrow spectrum illumination is desired. Typically, the light source is a coherent light source, such as a laser, or laser diode, although other light sources, such as LEDs, lamps or other available light sources are also optionally employed. In the case of a fluorescent detector, excitation light, e.g., light of appropriate wavelength to excite both reference and function labels, from the light source 602 is directed at the analysis channel 614, e.g., disposed in microfluidic device 612, via an optical train that includes optional lens 604, beam splitters 606 and 608 and objective lens 610. Upon excitation of both the reference and function labels present in channel 614, e.g., associated with cells in the channel, the emitted fluorescence is gathered through the objective lens 610 and passed through beam splitter 608. A portion of the emitted fluorescence is passed through a narrow band pass filter 616 which passes light having a wavelength approximately equal to the excitation maximum (the emitted fluorescence) of one of the two labels, while filtering out the other label's fluorescence, as well as any background excitation light. Another portion of the emitted fluorescence is passed onto beam splitter 606 which directs the fluorescence through narrow band pass filter 620, which passes light having the wavelength approximately equal to the emission maximum of the other label group. One or more of beam splitters 608 and 606 are optionally substituted with dichroic mirrors for separating the label fluorescence and/or any reflected excitation light. Detectors 618 and 622 are typically operably coupled to a computer which records the level of detected light as a function of time from the beginning of the assay.

As described in greater detail herein, in some instances, electrokinetic material transport systems are used to direct one or more of the flow of cell suspensions, the injection of test compounds, and other material movement parameters. In such cases, the overall system used in performing the assay will typically include an appropriate controller and interface for controlling such electrokinetic material transport. Typically, such transport systems include one or more electrical power supplies that are operably coupled to the termini of the channels in a microfluidic device, e.g., as described in greater detail below. The connection of the power supply(ies) with the channels is typically accomplished via electrodes placed into reservoirs at the termini of the channels, which electrodes are coupled to the power supply(ies). The power

supply(ies) then delivers appropriate voltage levels to the various electrodes to yield a desired flow pattern within the channels of the device, e.g., flowing the cell suspension and periodically injecting a test compound. The power supply is typically linked to an appropriately programmed computer which directs the application of voltages in accordance with a user selected flow profile.

B. Assay Devices

As noted above, the assays of the present invention are carried out within fluidic channels, along which the cell suspensions are flowed. In some cases, the channels may simply be present in a capillary tube, e.g., a glass, fused silica, quartz or plastic capillary. The capillary channel is fluidly coupled to a source of the suspension of cells, which are then flowed along the capillary channel. In particularly preferred aspects, the channel is integrated into the body structure of a microfluidic device. As used herein, the term "microfluidic" generally refers to one or more fluid passages, chambers or conduits which have at least one internal cross-sectional dimension, e.g., depth, width, length, diameter, etc., that is less than 500μm, and typically between about 0.1 μm and about 500 μm.

In the devices of the present invention, the microscale channels or chambers preferably have at least one cross-sectional dimension between about 0.1 μm and 200 μm, more preferably between about 0.1 μm and 100 μm, and often between about 0.1 μm and 50 μm. Accordingly, the microfluidic devices or systems prepared in accordance with the present invention typically include at least one microscale channel, usually at least two intersecting microscale channels, and often, three or more intersecting channels disposed within a single body structure. Channel intersections may exist in a number of formats, including cross intersections, "T" intersections, or any number of other structures whereby two channels are in fluid communication.

The body structure of the microfluidic devices described herein typically comprises an aggregation of two or more separate layers which when appropriately mated or joined together, form the microfluidic device of the invention, e.g., containing the channels and/or chambers described herein. Typically, the microfluidic devices described herein will comprise a top portion, a bottom portion, and an interior portion, wherein the interior portion substantially defines the channels and chambers of the device.

Figure 3 illustrates a two-layer body structure 300, for a microfluidic device. In preferred aspects, the bottom portion of the device 302 comprises a solid substrate that is

substantially planar in structure, and which has at least one substantially flat upper surface 304. A variety of substrate materials may be employed as the bottom portion. Typically, because the devices are microfabricated, substrate materials will be selected based upon their compatibility with known microfabrication techniques, e.g., photolithography, wet chemical etching, laser ablation, air abrasion techniques, injection molding, embossing, and other techniques. The substrate materials are also generally selected for their compatibility with the full range of conditions to which the microfluidic devices may be exposed, including extremes of pH, temperature, salt concentration, and application of electric fields. Accordingly, in some preferred aspects, the substrate material may include materials normally associated with the semiconductor industry in which such microfabrication techniques are regularly employed, including, e.g., silica based substrates, such as glass, quartz, silicon or polysilicon, as well as other substrate materials, such as gallium arsenide and the like. In the case of semiconductive materials, it will often be desirable to provide an insulating coating or layer, e.g., silicon oxide, over the substrate material, and particularly in those applications where electric fields are to be applied to the device or its contents.

In additional preferred aspects, the substrate materials will comprise polymeric materials, e.g., plastics, such as polymethylmethacrylate (PMMA), polycarbonate, polytetrafluoroethylene (TEFLON™), polyvinylchloride (PVC), polydimethylsiloxane (PDMS), polysulfone, polystyrene, polymethylpentene, polypropylene, polyethylene, polyvinylidine fluoride, ABS (acrylonitrile-butadiene-styrene copolymer), and the like. Such polymeric substrates are readily manufactured using available microfabrication techniques, as described above, or from microfabricated masters, using well known molding techniques, such as injection molding, embossing or stamping, or by polymerizing the polymeric precursor material within the mold (See U.S. Patent No. 5,512,131). Such polymeric substrate materials are preferred for their ease of manufacture, low cost and disposability, as well as their general inertness to most extreme reaction conditions. Again, these polymeric materials may include treated surfaces, e.g., derivatized or coated surfaces, to enhance their utility in the microfluidic system, e.g., provide enhanced fluid direction, e.g., as described in U.S. Patent Application Serial No. 08/843,212, filed April 14, 1997, and which is incorporated herein by reference in its entirety for all purposes.

The channels and/or chambers of the microfluidic devices are typically fabricated into the upper surface 304 of the bottom substrate or portion 302, as microscale grooves or indentations 306, using the above described microfabrication techniques. The top portion or

substrate 308 also comprises a first planar surface 310, and a second surface 312 opposite the first planar surface 310. In the microfluidic devices prepared in accordance with certain aspects of the methods described herein, the top portion 308 also includes a plurality of apertures, holes or ports 314 disposed therethrough, e.g., from the first planar surface 310 to the second surface 312 opposite the first planar surface.

The first planar surface 310 of the top substrate 308 is then mated, e.g., placed into contact with, and bonded to the planar surface 304 of the bottom substrate 302, covering and sealing the grooves and/or indentations 306 in the surface of the bottom substrate, to form the channels and/or chambers (i.e., the interior portion) of the device at the interface of these two components. Bonding of substrates is typically carried out by any of a number of different methods, e.g., thermal bonding, solvent bonding, ultrasonic welding, and the like.

The holes 304 in the top portion of the device are oriented such that they are in communication with at least one of the channels and/or chambers formed in the interior portion of the device from the grooves or indentations in the bottom substrate. In the completed device, these holes function as reservoirs for facilitating fluid or material introduction into the channels or chambers of the interior portion of the device, as well as providing ports at which electrodes may be placed into contact with fluids within the device, allowing application of electric fields along the channels of the device to control and direct fluid transport within the device. In many embodiments, extensions are provided over these reservoirs to allow for increased fluid volumes, permitting longer running assays, and better controlling fluid flow parameters, e.g., hydrostatic pressures. Examples of methods and apparatuses for providing such extensions are described in U.S. Application No. 09/028,965, filed February 24, 1998, and incorporated herein by reference.

In many embodiments, the microfluidic devices will include an optical detection window disposed across one or more channels and/or chambers of the device. Optical detection windows are typically transparent such that they are capable of transmitting an optical signal from the channel/chamber over which they are disposed. Optical detection windows may merely be a region of a transparent cover layer, e.g., where the cover layer is glass or quartz, or a transparent polymer material, e.g., PMMA, polycarbonate, etc. Alternatively, where opaque substrates are used in manufacturing the devices, transparent detection windows fabricated from the above materials may be separately manufactured into the device.

In many aspects, it is desirable to provide the interior surfaces of the channels with an appropriate treatment to prevent the adhesion of cells to that surface. For example, in the case

of glass or other highly charged channel surfaces, some cell types may have a tendency to stick to the channel surfaces, interfering with the flowing of cells through the channels. For example, in the case of mammalian cell based assays, many mammalian cell types are particularly adherent to certain types of surfaces, e.g., glass and some plastics. Accordingly, in some embodiments, it is desirable to treat or coat the interior surfaces of the channels to prevent cell adhesion. A variety of surface treatments are optionally employed to accomplish this goal. For example, charge masking coatings such as polyols (e.g., polyvinylalcohol (PVA)) polyethyleneimine (PEI), polyethylene glycol (PEG), polyacrylamides (e.g., polyacrylamide, polymethylacryalamide, polydimethacrylamide, and the like), carbohydrates such as polysucrose (ficoll), polyglucose (dextran and cellulose), and polytetrafluoroethylene (Teflon™), etc.. Alternatively, covalent surface treatments are also optionally used to prevent surface adhesion of cells, such as silanization (e.g., using dimethyl or dichlorosilane) of glass or plastic surfaces. Other surface treatments are generally described above, with reference to device fabrication techniques, above.

The flowing of the suspension of cells along the analysis channels of the devices described herein is optionally carried out by a number of mechanisms, including pressure based flow, electrokinetic flow, or mechanisms that utilize a hybrid of the two. In a first preferred aspect, a pressure differential is used to flow the suspension of cells along the analysis channel. Application of a pressure differential along the analysis channel is carried out by a number of means. For example, in a simple passive aspect, the cell suspension is deposited in a reservoir at one end of the analysis channel and at a sufficient volume or depth, that the cell suspension creates a hydrostatic pressure differential along the length of the analysis channel, e.g., by virtue of its having greater depth than a reservoir at an opposite terminus of the channel. The hydrostatic pressure then causes the cell suspension to flow along the length of the channel. Typically, the reservoir volume is quite large in comparison to the volume or flow through rate of the channel, e.g., 10 μl reservoirs, vs. 1000 μm^2 channel cross-section. As such, over the time course of the assay, the flow rate of the cell suspension will remain substantially constant, as the volume of the reservoir, and thus, the hydrostatic pressure changes very slowly. Applied pressure is then readily varied to yield different cell suspension flow rates through the channel. In screening applications, varying the flow rate of the cell suspension is optionally used to vary the incubation time of the cells with the test compound. In particular, by slowing the cells flow rate along the channel, one can effectively lengthen the amount of time between intrroduction of test compounds and detection

of their effects. Alternatively, analysis channel lengths, detection points, or test compound introduction points are varied in fabrication of the devices, to vary incubation times.

In many applications, it may be desirable to provide relatively precise control of the flow rate of the cell suspension, e.g., to precisely control incubation times, etc. As such, in many preferred aspects, flow systems that are more active than hydrostatic pressure driven systems are employed. For example, the cell suspension may be flowed by applying a pressure differential across the length of the analysis channel. For example, a pressure source is applied at the cell suspension reservoir at one end of the analysis channel, and the applied pressure forces the suspension through the channel. The pressure source can be pneumatic, e.g., a pressurized gas, or alternatively can be a positive displacement mechanism, i.e., a plunger fitted into a cell suspension reservoir, for forcing the cell suspension through the analysis channel. Alternatively, a vacuum source is applied to a reservoir at the opposite end of the channel to draw the suspension through the channel. Pressure or vacuum sources may be supplied external to the device or system, e.g., external vacuum or pressure pumps sealably fitted to the inlet or outlet of the analysis channel, or they may be internal to the device, e.g., microfabricated pumps integrated into the device and operably linked to the analysis channel. Examples of microfabricated pumps have been widely described in the art. See, e.g., published International Applicatin No. WO 97/02357.

In alternate aspects, other flow systems are employed in transporting the cellular suspension through the analysis channel. One example of such alternate methods employs electrokinetic forces to transport the cells. Electrokinetic transport systems typically utilize electric fields applied along the length of channels that have a surface potential or charge associated therewith. When fluid is introduced into the channel, the charged groups on the inner surface of the channel ionize, creating locally concentrated levels of ions near the fluid surface interface. Under an electric field, this charged sheath migrates toward the cathode or anode (depending upon whether the sheath comprises positive or negative ions) and pulls the encompassed fluid along with it, resulting in bulk fluid flow. This flow of fluid is generally termed electroosmotic flow. Where the fluid includes a cell suspension, the cells are also pulled along. A more detailed description of controlled electrokinetic material transport systems in microfluidic systems is described in published International Patent Application No. WO 96/04547, which is incorporated herein by reference.

In accordance with the methods and systems of the present invention, a voltage gradient is applied along the length of the analysis channel, which includes an inner surface that

comprises a surface potential. In the case of glass channels, the surface typically comprises hydroxyl groups. In aqueous systems, e.g., in the presence of the suspension of cells, these hydroxyl groups deprotonate creating a sheath of positive ions in the fluid near the fluid/surface interface. The voltage gradient is typically applied by placing an electrode at different ends of the analysis channel, e.g., in the reservoirs at the termini of the analysis channel. A voltage difference is then applied between the two electrodes, to cause the suspension of cells to electroosmotically flow along the length of the channel, toward the lower potential.

Flow rates are typically varied by increasing or decreasing the voltage gradient along the channel. As described in greater detail below, electrokinetic transport is optionally used to inject test compounds into the flowing cell suspension.

In many instances, a completely electrokinetic transport system is not ideal for use in transporting cell suspensions. In particular, in many cases, the elevated electric fields used in electrokinetic transport can result in permeation of the cells' membranes, e.g., electroporation. Such electroporation of cells can lead to reduced viability of cells, or at the very least, a leaking of cellular contents including reference label. Accordingly, electrokinetic transport systems are optionally used which do not expose the cell suspension to the electric field, which drives the flowing suspension of cells. In particular, and as described in greater detail below with reference to Figure 5, the devices and systems of the invention optionally employ electroosmotic pressure pumps that utilize an electric field away from the flowing suspension of cells, to drive the flow of the suspension of cells. Electroosmotic pressure pumps are generally described in U.S. Application No. 08/937,958, filed September 25, 1997, which is incorporated herein by reference.

In alternative aspects, flow of the cell suspension is driven by inertial forces. In particular, the analysis channel is optionally disposed in a substrate that has the conformation of a rotor, with the analysis channel extending radially outward from the center of the rotor. The cell suspension is deposited in a reservoir that is located at the interior portion of the rotor and is fluidly connected to the channel. During rotation of the rotor, the centripetal force on the cell suspension forces the cell suspension through the analysis channel, outward toward the edge of the rotor. Multiple analysis channels are optionally provided in the rotor to perform multiple different analyses. Detection of the function and reference labels is then carried out by placing a detector under the spinning rotor and detecting the signal as the analysis channel passes over the detector. Examples of rotor systems have been previously described for performing a number of

different assay types. See, e.g., Published International Application No. WO 95/02189. Test compound reservoirs are optionally provided in the rotor, in fluid communication with the analysis channel, such that the rotation of the rotor also forces the test compounds into the analysis channel.

A simplified example of a device used in practicing the assay and screening methods described herein, is schematically illustrated in Figure 4. As shown, the device 400 adopts a planar body structure 402 that was described with reference to Figure 3, above. The device 400 includes a first reservoir 404 disposed in the body structure 402, and into which the suspension of cells is placed. A plurality of channels 406-428 are provided in the body structure in fluid communication with the cell suspension reservoir 404. As shown, each channel has one terminus in fluid communication with reservoir 404, and the other terminus in communication with a separate reservoir 430-452, respectively. Separate reservoirs for each channel are optional, and are readily replaced with a single receiving or waste reservoir, similar to cell suspension reservoir 404. A source of test compound 454-476 is optionally provided in fluid communication with each separate analysis channel. In operation, the cell suspension is deposited in reservoir 404 and permitted or caused to flow along each of the analysis channels 406-428, and past detection window 480, by one of the flow means described herein, e.g., pressure flow, electrokinetic flow, intertial flow, etc.

Test compounds are placed into the test compound reservoirs 454-476, and permitted or caused to flow into their respective analysis channels. Again, these test compounds may be allowed to flow passively into their respective analysis channels, or may be actively injected, e.g., through pressure or electrokinetic flow means. As the cell suspension passes the detection window 480, the amount of reference and function label is detected in each channel, by one or multiple detectors disposed adjacent to the detection window. Such detectors may include scanning fluorescent detectors that scan across the full length of the detection window, covering several analysis channels in relatively short times, i.e., galvanometer scanners, track scanners and the like. Optionally, linear array scanners are used in the detection process, i.e., incorporating linear arrays of CCDs.

In the performance of cell rolling assays, it will appreciated that a portion of all or most of the analysis channels 406-428 are optionally provided with one or more different ligands immobilized therein, to assay for cell rolling or firm adhesion. For example, the portion of the

channels observed through the detection window 480 typically will include the ligand coated surface.

An alternative example of a device is schematically illustrated in Figure 5, from a top and side view (panels A and B, respectively). As shown, the device 500 includes an analysis channel 506 disposed in a body structure 502. The analysis channel 506 includes a detection point or window 524 disposed over the analysis channel 506. Typically, the body structure is fabricated from transparent material. As such, the detection point or window can be located over the analysis channel at virtually any point along that channel, depending upon the assay that is to be performed, the amount of incubation time desired, etc. A reservoir 514 is also provided disposed in the substrate, into which is placed a suspension of cells. The cell suspension reservoir 514 is in fluid communication with the analysis channel 506 that is also disposed in the body structure. The reservoir may be directly connected to the analysis channel or it may be connected through an additional channel, e.g., channel portion 512. The device also includes an external sample accessing capillary 504, through which different test compounds are introduced into the analysis channel. Test compounds or samples may be drawn up into the capillary channel and the channel network of the device itself using the same driving forces used to drive materials through the channels of the device, e.g., applied vacuum, electrokinetics, etc.

Examples of accessing capillaries that are particularly suitable for incorporation into devices of the type shown are pipettor capillaries, e.g., as described in commonly owned published International Patent Application No. 98/00705, which is incorporated herein by reference. Typically, these pipettors comprise capillary channels that have electrodes disposed at or proximal to their external terminus (the capillary terminus not coupled to the integrated channel network), such that the electrode is in contact with a source of sample or test compound while the end of the capillary is immersed in the sample material. An electric field is then generated between the external electrode and an electrode in communication with the integrated channel structure of the device to electrokinetically drive materials through the capillary into the channel network of the device. These external sample accessing capillaries are then placed in fluid communication with different test compound or sample sources, e.g., different wells in a multiwell plate, by moving the capillary end into each of the wells or sources. This ability to move from sample source to sample source, sipping small amounts of sample allows the capillary to be in "selective fluid communication" with each of the sample sources.

In operation, a suspension of cells is placed into reservoir 514, and the suspension flows into the analysis channel via channel 512. Flowing of the suspension of cells from the suspension reservoir 514, into the analysis channel 506, past the detection window 524, and into the waste reservoir 518, is carried out by any of the flow systems described above, e.g., electrokinetic, pressure based, or the like. As shown, the hydrostatic pressure of the cell suspension in the reservoir 510 is used to flow the cell suspension into the analysis channel 506.

Different test compounds are then obtained from libraries of compounds, e.g., disposed in multiwell plates, e.g., well 530, and introduced into the analysis channel by immersing the sample accessing capillary into the different compound sources or wells and drawing an aliquot of the test compound into the capillary. The aliquot of test compound is subsequently moved into the analysis channel, where it is mixed with the suspension of cells, and the effect of the test compound on the cells, if any, is measured at the detection point.

As noted above, in certain aspects, it may be desirable to avoid subjecting the suspension of cells to electric fields required for electrokinetically transporting the cells. Similarly, electric fields required for introducing test compounds through, e.g., an electropipettor may also have detrimental effects on the cells in the system. Accordingly, in certain aspects, samples are drawn into the system using non-electrokinetic forces, e.g., pressure. Alternatively, the pipettor is configured to function as an electroosmotically driven micropump, for mixing test compounds with cells, and for flowing the cells along the analysis channel.

In such cases, the device shown in Figure 5 is readily adapted to such flow systems. In particular, in the device 500 illustrated in Figure 5, electrokinetic introduction of test compounds is accomplished by providing a voltage gradient between the source of test compounds, e.g., fluid well 530, and the integrated channel structure in the interior of the device 500. As shown, the voltage gradient is applied between the fluid well 530 and reservoir 510, such that the test compound is flowed from well 502 into the capillary 504 and into channel portion 506a. Power supply 526 supplies the voltage gradient between the test compound source 530 and optional reservoir 510 via electrodes 528 and 530, respectively. In order to prevent the test compounds from electrokinetically flowing through channel 506a, into optional channel 508 and into reservoir 510, channel 508 is typically configured to present no electroosmotic potential when exposed to an electric field, i.e., electroosmotic flow is substantially absent within the fluid-filled channel, when exposed to an electric field. The elimination of electroosmotic flow in channel 508 is optionally accomplished by a number of methods. For example, channel 508 may

be treated differently from channel 506a, so as to mask any surface charge in the channel that might give rise to electroosmotic flow. A variety of surface coatings have been described in the capillary electrophoresis art for eliminating electroosmotic flow in silica capillaries, and such methods are equally applicable to the devices described herein. See, e.g., Lopez et al., J. Am. Chem. Soc. (1993) 115:10774, Bruin et al., J. Chromatog. (1989) 471:429, Townes et al. J. Chromatog. (1990) 516:69. Other surface treatments are also optionally employed, e.g., covalent modification of functional groups at the surface of the channel, such as silanization reactions.

When used to perform, e.g., cell rolling assays, typically at least a portion of channel 506 is provided with an appropriate binding group immobilized to the interior channel surface. Detection window 524 is then used to obtain images of cells traveling along the channel 506. Alternatively, a second detection window (not shown) is provided upstream of detection window 524, for first detecting the cells or group of cells that are being monitored. The cells or groups of cells are then detected at window 524, and their travel time from one point to the other is determined. The velocity f the cells through the binding region of the channel is then used as a measure of the relative level of cellular adhesion to the binding moieties in the channel. The various test compounds are then added to determine their effects, if any, on that velocity.

Alternatively, optional channel 508 is optionally provided with an ion permeable barrier or plug, e.g., a salt bridge, which allows current, but not fluid, to pass. Such plugs include, e.g. gel plugs that are polymerized in situ, e.g., through photopolymerization, and which prevent fluid flow, while permitting the passage of current. Such a configuration permits the sample accessing capillary 504, and channel 506a to operate in conjunction with the electrical control system to create an electroosmotically driven pressure pump. Examples of electroosmotic pressure pumps that are particularly useful in this regard are described in commonly owned U.S. Patent Application No. 08/937,958, filed September 25, 1997, which is incorporated herein by reference. Similarly, the use of salt bridges in microfluidic systems is described in published International Application No. 98/00231, which is incorporated herein by reference.

Although the masked surface charge prevents fluid from flowing along channel 508, it does not prevent the tapping of the electrical current from channel 506a into channel 508. The cell suspension is deposited in reservoir 514 and allowed to flow along the analysis channel 506, in the absence of any electrical current. Specifically, by tapping the current upstream of the cell suspension, e.g., into reservoir 510 via channel 508, it prevents the cell suspension from

being exposed to the electrical current. Because fluid cannot readily flow along channel 508, the pressure induced by the electroosmotic pumping of the fluid within capillary 504 and channel 506a forces the fluid, including the test compound, to flow into the analysis channel 506. The pressure resulting from the electroosmotic pumping of the electropipettor also ensures that the cell suspension flows along the analysis channel in the direction from the suspension reservoir 514 to the waste reservoir 518, and past detection window 524. In alternative aspects, the electroosmotic pressure pump is provided within the interior portion of the device 500. For example, optional channel 520 and reservoir 522 may be provided in fluid communication with channel 506a downstream of the detection window, e.g., at a point between the detection window and channel 516/reservoir 518. Channel 520 and reservoir 522 are provided in place of channel 508 and reservoir 510, and perform the same function of these elements in the same manner. Specifically, electrode 530 may be contacting the fluid in reservoir 522, while electrode 528 contacts the fluid in reservoir 518. The voltage gradient is then applied to such that fluid is electroosmotically drawn into reservoir 518. Because channel 520 is configured so as to prevent electroosmotic flow of fluid, e.g., as described for channel 508, above, the fluid drawn into reservoir 518 is pulled from analysis channel 506. Again, this type of electroosmotic pressure pump/aspirator is described in U.S. Patent Application No. 08/937,958, filed September 25, 1997, incorporated herein by reference.

Although illustrated as a single channel and accessing capillary, it will be readily appreciated that these aspects may be provided as multiple parallel analysis channels and accessing capillaries, in order to substantially increase the throughput of the system. Specifically, single body structures may be provided with multiple parallel analysis channels coupled to multiple sample accessing capillaries that are positioned to sample multiple samples at a time from sample libraries, e.g., multiwell plates. As such, these capillaries are generally spaced at regular distances that correspond with the spacing of wells in multiwell plates, e.g., 9 mm centers for 96 well plates, 4.5 mm for 384 well plates, and 2.25 mm for 1536 well plates.

Thus, the present invention provides for the use of cells comprising a reference label and a function label to assay for a relative function level of the cells. Typically, in this use, the cells are flowed along a first channel during the assay, which is typically a microscale channel. The use of these cells is in assaying for the cellular function in the presence and absence of test compounds.

Relatedly, the invention provides for the use of a microfluidic channel to assay for a cellular function comprising flowing cells along the channel, the cells comprising a reference label and a function label, and detecting a level of reference label and a level of function label, a ratio of function label to function label providing a relative level of the cellular function.

The present invention is further illustrated with reference to the following non-limiting examples.

EXAMPLES

The following non-limiting examples are provided as being illustrative of the methods, devices and systems of the present invention. The full scope of the present invention, however, is defined only by the literal and equivalent scope of the appended claims.

Example 1: Dual Label Cell Function Assay Methods

The assay methods of the invention were demonstrated in CHO and THP-1 cells, screened against known agonists of calcium transport for these cells.

Cell Culture- Cells (CHO or THP-1) were obtained from the ATCC. THP-1 cells were cultured in RPMI 1640 media containing 10% fetal bovine serum (FBS), sodium pyruvate (1 mM), L-glutamine (2 mM), Penicillin-G/Streptomycin (100 u/ml, 100 µg/ml), beta-mercaptoethanol (50 µM), HEPES buffer, pH 7.4 (10mM). CHO cells were cultured in Ham's F12 media containing 10%FBS, L-glutamine (2 mM), and Penicillin-G/Streptomycin (100u/ml/100µg/ml). The cells were maintained by splitting every 3-4 days.

Dye Loading of Cells- The cells were loaded with Fluo-3-AM dye (Molecular Probes) using a 4 µM solution of the dye in Hank's Balanced Salt Solution (HBSS) containing calcium and magnesium but lacking phenol red. Added to the HBSS were 1% FBS, 2.5 mM probenecid, 30 mM HEPES, pH 7.0, and 0.05% pluronic acid. The cells were incubated in the dye containing solution at approximately, 5-9 x 10^6 cells/5 ml for 50 minutes in a CO_2 incubator at 37° C. At the end of the incubation, Syto-17, a nucleic acid staining dye (Molecular Probes), was added at 1µM, and cells were further incubated for 10 minutes at room temperature. The dye loaded cells were washed free of excess dye using two washes by centrifugation at 300 x g and resuspension in 5 mls of HBSS containing bovine serum albumin (BSA, 1mg/ml), probenecid (2.5 mM), and HEPES buffer, pH 7 (20 mM). The washed cells were resuspended into Cell Assay Medium. The assay medium for CHO cells was HBSS containing bovine serum

albumin (BSA, 1mg/ml), probenecid (2.5 mM), and HEPES buffer, pH 7.4 (30 mM) and PVA (0.001%). The resuspended cells were then mixed with ficoll-hypaque (2:1 cells:hypaque). For THP-1 cells, the assay medium contained sucrose (8.5%), dextrose (0.3%), HEPES buffer, pH 7.5 (30 mM), NaCl (16 mM), $MgCl_2$ (1 mM), $CaCl_2$ (1 mM), BSA (10 mg/ml), Probenecid (2.5 mM), and polyvinyl alcohol (PVA 90,000 avg. mol.wt., 0.001%). The final density of the cell suspension was 10 x 10^6 cells/ml. The cells were stored at room temperature until used for testing.

On-Chip Cell Analysis - A microfluidic device having the channel geometry shown in Figure 8 was used in performing the assay. The channels of the device were rinsed with PVA at 0.2% in cell culture grade water by allowing the solution to wick into the dry device by capillary action and aspirating additional solution through the channels. The PVA solution was displaced with Cell Assay Medium by aspiration through the channels of the device.

The fluid path which the cells followed during the testing was from well #5 to well #3 in the device. The cells were applied in 10 µl to well #5 and the test sample was applied in 10 µl to well #6. Both cells and sample flowed into the fluid channel moving toward well #3. Cell Assay Medium (10 µl) was added to well #8, and well #3 was empty. The fluid continuously moved down the hydrodynamic gradient from wells 5, 6, and 8 into well 3. The ratio of mixing was estimated to be 1/3 sample, 1/6th Assay Medium, and 1/2 cell volume from the measurement of an indicator fluorescent dye before and after mixing with the cell and medium fluid streams. During the time course of the typical test (10-20 minutes), the fluid heights do not change significantly; therefore, the pressure gradients do not change during the course of the test. Some variation in flow velocity was observed during the testing which appeared to be due to the partial filling of the empty waste well causing decreased wicking at the channel exit, in-turn causing decreased flow rate.

The fluorescence of the cells was measured using two PMT's on a Nikon inverted microscope and fluorescein/texas red filter sets (Fluo-3: Ex. max=506, Em. max=526 & Syto-17: Ex. max=621, Em. max=634 nm) for two color analysis. Using the combination of Fluo-3 and Syto-17 dyes, there was no interference by fluorescence quenching of one dye's emission by the other dye. It was, therefore, possible to measure these two indicators simultaneously. The Syto-17 stains all THP-1 or CHO cells similarly because it is permeable to the cell membrane and selective for both RNA and DNA binding. Syto-17 was therefore an indicator of cell density in the reading area. Fluo-3's fluorescence is enhanced by calcium, and, therefore, is a measure of

intracellular calcium concentration. The cellular fluorescence was measured in a reading window covering 50 μm of the channel length and the entire width of the channel (100 μm) at a distance down-stream of the point of sample addition that was traversed by cells in 10 seconds. The fluorescence was monitored during the test for 100 seconds, during which time approximately 3,300 cells pass the detection window. The sampling rate of the PMT's was 20 hertz and the time constant of sampling was 5 msec., generating about 2000 data points within the 100 second reading interval.

Example 2: Application of Dual Label Cell Function Assay in Dose Response Determination of CHO cells to Ionomycin Addition

CHO cells prepared as described above, were flowed through the analysis channel (between wells 5 and 3) and were contacted with different concentrations of ionomycin, a calcium ionophore (0, 1 μM, 3 μM, 10 μM and 30 μM) that were introduced from well 6, in separate experiments. Scatter plots from each of these separate experiments are shown in Figure 7A-7E. As shown, the amount of intracellular calcium within the cells was readily detected with increasing concentrations of ionomycin. In particular, the slope of the best fit line for the scatter plots increases with increasing ionomycin concentration.

Figure 7F shows a dose response curves for ionomycin in CHO cells, plotting the best fit slope (from Figures 7A-7E) versus ionomycin concentration. The slope corresponds to the ratio of calcium modulated fluorescence to cell number and is, therefore, a measure of intracellular calcium concentration. As expected, the intracellular calcium concentration and slope increase with increasing concentrations of ionomycin. Accordingly, these experiments dramatically demonstrate the efficacy of the dual label methods of the present invention for monitoring relative levels of a particular cellular function, i.e., that result in changes in intracellular calcium concentration.

Example 3: Application of Dual Label Cell Function Assay in Dose Response Determination of THP-1 Cells to UTP

THP-1 Cells are acute monocytic leukemia cells that can be propagated in culture and maintain the expression of purinergic, P2Y receptors. These G protein-coupled receptors are activated by UTP, ATP, UDP, & ADP and are coupled to cellular Gq mediated calcium signaling

pathways. THP-1 cells loaded with a calcium sensitive dye, Fluo-3, were used in this portion of the experiment, and the calcium response of these cells to UTP was detected.

Figure 9 shows the data collected from tests using no UTP (lower group of plotted points) and another test using 30 μM UTP as sample (upper group of plotted points). The scatter plot indicates that the ratio of the Fluo-3 and Syto-17 fluorescence (the slope determined by linear regression) changes with treatment with UTP, an agonist for the P2Y receptor on THP-1 cells.

Results using UTP Treatment of THP-1 Cells- A Concentration Response Curve was generated using the On-Chip Cell Analysis system, treating the THP-1 cells with increasing concentrations of UTP, and is shown in Figure 10. Kinetic data can also be generated using the On-Chip Cell Analysis System by moving the fluorescence measurement point closer or further from the point of sample addition. This changes the time of incubation with test sample prior to measurement of calcium fluorescence. A shown in Figure 11, using THP-1 cells and 10 μM UTP treatment, the calcium response rapidly peaks within 5 seconds of sample introduction.

Example 4: TUNEL Assay and Annexin-V assay for Apoptosis Detection

A TUNEL assay (TdT (Terminal deoxynucleotidyl transferase)- mediated dUTP Nick End Labeling) was used to measure cellular apoptosis in accordance with the above-described methods and systems. In a TUNEL Assay, damaged DNA is labeled with fluorescinated nucleotides. Terminal deoxynucleotidyl transferase (TdT) binds to exposed 3' ends of DNA fragments generated in response to apoptotic signals and catalyzes the addition of fluorescein-labeled deoxynucleotides. U937 cells were treated with Campthotecin to induce apoptosis. The cells were then harvested and fixed with 4% formaldehyde and stored in 80% ethanol at +4°C until labeling is performed.

The cells were rehydrated in TBS buffer. They were then treated with 20 μg/ml of proteinase K for 5 minutes and resuspended in equilibration buffer. The cells were incubated in a working TdT labeling reaction mixture from Oncogene Research Products containing TdtT enzyme and FragEL TdT labeling reaction mix. The cells were resuspended in TBS and then counterstained with a DNA dye (Syto 62 DNA dye at 1 mM for 10 minutes RT) to obtain a total cell count.

After washing the cells in TBS, they were resuspended in Hanks' Balanced Salt Solution with 10% OPTIPREP at 5 x 10^6/ml and loaded in a microfluidic device having the

channel geometry shown in Figure 8, for analysis. Fluorescence was detected using 488nm excitation, while emission was read at 525 nm and 680 nm.

Figure 12 illustrates a control analysis, e.g., U937 cells not treated to induce apoptosis. The bottom line corresponds to the Syto-62, which indicates the mere presence of cells, whereas the top line corresponds to the fluorescein end labeled nucleic acids. Apoptotic cells would yield a peak on both he upper and lower lines, indicating the presence of an apoptotic cell. As can be seen, there are substantially no discernible peaks in the upper line, indicating a lack of apoptosis in the control. Figure 13 represents U937 cells treated with Campthotecin to induce apoptosis. As can be seen, corresponding peaks are seen on both the lower and upper lines, indicating the presence of apoptotic cells.

In a Annexin-V assay, change in the outer membrane of apoptotic cells is detected. A membrane component, phosphatidylserine (PS) is translocated to the outer layer. Annexin-V binds to PS and is conjugated to biotin to allow secondary reactions to fluorescently labeled Streptavidin for detection. U937 cells were treated with Campthotecin to induce apoptosis. The cells were harvested and washed in phosphate buffered saline (PBS). Cells were incubated with Annexin-V-Biotin (Boehringer Mannheim) in HEPES buffer, and then incubated with 5 μg streptavidin-Cy5 (Amersham) and 1mM of Calcein-AM dye in HEPES buffer. The HEPES buffer solution contained 10mM HEPES, pH 7.4; 140 mM NaCl; and 5 mM CaCl2. The cells were washed and resuspended in HEPES buffer with 8.5% sucrose and loaded in a microfluidic device for analysis. Fluorescence is detected using excitation at 488 nm and 635 nm, and emission is read at 525 nm and 682 nm.

Figure 14 indicates analysis of U937 cells that were not treated to induce apoptosis. In this case, the bottom line indicates live cell count (Calcein), while the top line indicates the presence of apoptotic cells (Annexin-V-Cy5). As can be seen, a few apoptotic cells are present within the control experiment. Figure 15, on the other hand, shows an analysis of the same cells treated with Campthotecin to induce apoptosis. As can be seen, the top trace includes a much greater number of peaks representing apoptotic cells, and particularly as a percentage of total cells in the analysis, e.g., as compared to the lower line.

All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. Although the present invention has been described in some detail by way of illustration and example for purposes of clarity and

understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

What is claimed is:

1. A method of measuring a function of cells, comprising:

flowing a suspension of cells along a first fluid channel, the cells having a first detectable property associated therewith, and wherein the cells produce a second detectable property upon activation of the function of the cells, the first and second detectable properties being distinguishable from each other;

measuring levels of the first and second detectable properties;

comparing the level of second detectable property to the level of first detectable property to determine a relative function of the cells.

2. The method of claim 1, wherein the flowing step comprises applying a pressure differential along the first fluid channel to flow the suspension of cells along the first fluid channel.

3. The method of claim 2, wherein the pressure differential is applied by applying an elevated pressure at one terminus of the first fluid channel.

4. The method of claim 2, wherein the pressure differential is applied by applying a pressure source or vacuum source to one terminus of the first fluid channel.

5. The method of claim 2, wherein the pressure differential is applied through hydrostatic pressure of the suspension of cells at one terminus of the first fluid channel.

6. The method of claim 2, wherein the pressure differential is applied by positively displacing the suspension of cells in the first fluid channel.

7. The method of claim 1, wherein the flowing step comprises applying an electric field along a length of the first fluid channel, the electric field being sufficient to cause electrokinetic flowing of the suspension of cells along the first fluid channel.

8. The method of claim 1, wherein the flowing step comprises applying an inertial force to the suspension of cells to cause the suspension to flow along the first fluid channel.

9. The method of claim 1, wherein the first detectable property is substantially uniformly associated with cells in the suspension.

10. The method of claim 1, wherein the first detectable property is substantially uniformly associated with a distinct subset of cells in the suspension.

11. The method of claim 1, wherein the step of measuring the first and second detectable properties comprises measuring the first and second detectable properties from a plurality of cells in the suspension of cells, simultaneously.

12. The method of claim 11, wherein the cells in the suspension are blood cells, and the distinct subset of cells is selected from B cells, T cells, monocytes and neutrophils.

13. The method of claim 1, wherein the suspension of cells comprises a suspension of beads having cells adhered thereto.

14. The method of claim 1, wherein the first detectable property comprises a non-native label associated with the cells.

15. The method of claim 14, wherein the first detectable property comprises a label selected from a fluorophore, a chromophore, a colloidal label, and an electrochemical label.

16. The method of claim 14, wherein the first detectable property comprises a label associated with nucleic acids within the cells.

17. The method of claim 14, wherein the first detectable property comprises a label associated with membranes of the cells.

18. The method of claim 1, wherein the second detectable property comprises a non-native label, a signal level from the label increasing or decreasing upon activation of the function of the cells.

19. The method of claim 18, wherein the second detectable property comprises a second label selected from a fluorophore, a chromophore, a colloidal label and an electrochemical label.

20. The method of claim 18, wherein the second label comprises a fluorophore.

21. The method of claim 18, wherein the first and second detectable properties comprise first and second fluorophores, the first and second fluorophores being distinguishable from each other.

22. The method of claim 21, wherein the first and second fluorophores are distinguishable from each other based upon their absorption maxima, emission maxima or fluorescent lifetime.

23. The method of claim 1, wherein the function of the cells to be measured is selected from binding functions, transport functions, expression functions, apoptosis and viability.

24. The method of claim 23, wherein the function of the cells is a binding function, the binding function comprising binding of a first member of a specific binding pair to a second member of a specific binding pair, one of the first and second members being associated with the cells, and wherein the second detectable property comprises a label associated with a first member of a specific binding pair.

25. The method of claim 24, wherein the second detectable property comprises a label that is activated upon binding of the first member of the binding pair to the second member of the binding pair.

26. The method of claim 23, wherein the function label comprises a reporter gene product, the reporter gene being expressed upon binding of a first member of a specific binding pair with a second member of a specific binding pair associated with the cells.

27. The method of claim 24, wherein the function label increases or decreases in its level of detectability upon binding of the first member with the second member.

28. The method of claim 24, wherein the second detectable property comprises a fluorophore or chromophore associated with the first member of the specific binding pair.

29. The method of claim 24, wherein the first member of the specific binding pair comprises a ligand and the second member comprises a receptor associated with the cells, the ligand specifically interacting with the receptor.

30. The method of claim 29, wherein the receptor comprises a cell surface receptor.

31. The method of claim 30, wherein the receptor comprises a G-protein coupled receptor.

32. The method of claim 23, wherein the function of the cells to be determined comprises a transport function, and the second detectable property comprises a label associated with an intracellular indicator compound.

33. The method of claim 32, wherein the transport function is an ion transport function and the intracellular indicator comprises an ion specific indicator compound within the cells.

34. The method of claim 33, wherein the transport function is a proton transport function, and the intracellular indicator is an intracellular pH indicator.

35. The method of claim 34, wherein the intracellular pH indicator is selected from SNAFL, SNARF, BCECF, and HPTS.

36. The method of clam 33, wherein the ion transport function is selected from Ca^{++}, Na^{+}, K^{+}, Cl^{-}, or H^{+} transport functions.

37. The method of claim 36, wherein the ion transport function comprises a chloride transport function, and the intracellular indicator compound is selected from 6-methoxy-N-(sulfopropyl)quinolinium (SPQ), N-(sulfopropyl)acridinium (SPA), N-(6-methoxyquinolyl)acetic acid, and N-(6-methoxyquinolyl)acetoethyl ester.

38. The method of claim 36, wherein the ion transport function comprises a calcium transport function, and the intracellular indicator comprises 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA) or an analog thereof.

39. The method of claim 36, wherein the ion transport function comprises a calcium transport function and the intracellular indicator is selected from Fura-2, Fluo-2, Fluo-3, Fluo-4, Indo-1, and Calcium Green-2, or an analog thereof.

40. The method of claim 23, wherein the function of the cells to be measured is an expression of a selected macromolecular species, and the second detectable property is increased or decreased in response to the expression.

41. The method of claim 23, wherein the function of the cells to be determined is viability of the cells, and the second detectable property is increased or decreased in the presence of non-viable cells.

42. The method of claim 41, wherein the second detectable property comprises an ethidium bromide homodimer.

43. The method of claim 23, wherein the function of the cells to be monitored is apoptosis.

44. The method of claim 43, wherein the second detectable property comprises end labeled nucleic acids.

45. The method of claim 44, wherein the end labeled nucleic acids are fluoresceinated by a TUNEL method.

46. The method of claim 43, wherein the second detectable property comprises an indicator of a change in membrane composition resulting from apoptosis.

47. The method of claim 46, wherein the change in membrane composition comprises translocation of phosphatidylserine to an outer layer of the membrane.

48. The method of claim 47, wherein the second detectable property comprises Annexin-V labeled with a detectable moiety.

49. The method of claim 48, wherein the detectable moiety on the Annexin-V comprises biotin.

50. The method of claim 1, wherein the cells comprise a reporter construct, the reporter construct producing the second detectable property upon stimulation of a particular function of the cells.

51. The method of claim 50, wherein the reporter construct is not native to the cells.

52. The method of claim 1, wherein the function of the cells is determined in the presence of a test compound.

53. The method of claim 1, further comprising a step of introducing a test compound into the channel, wherein the function of the cells is determined in the presence of the test compound.

54. The method of claim 1, further comprising introducing a plurality of different test compounds, serially into the channel, wherein the function of the cells is separately determined in the presence of each of the different test compounds.

55. The method of claim 54, wherein the plurality of different test compounds comprise members of a pharmaceutical candidate library.

56. The method of claim 1, wherein the first and second detectable properties are measured at a point along the first channel.

57. The method of claim 1, wherein the first and second detectable properties are measured at more than one point along the first channel

58. The method of claim 1, wherein the second detectable property comprises an increase or decrease in a detectable label resulting from activation of the function of the cells.

59. The method of claim 1, wherein the first and second detectable properties are measured over time.

60. The method of claim 1, wherein the comparing step comprises determining a ratio of the second detectable property to the first detectable property, the ratio being indicative of the relative level of the function of the cells.

61. The method of claim 60, wherein the comparing step is carried out by a computer.

62. The method of claim 1, wherein the suspension of cells comprises from about $1X\ 10^5$ to about $1X\ 10^7$ cells/ml.

63. The method of claim 1, wherein the cells are selected from mammalian, insect, bacterial, fungal, yeast and plant cells.

64. The method of claim 62, wherein the cells comprise mammalian cells.

65. The method of claim 1, wherein in the flowing step, the first fluid channel comprises at least one microscale cross-sectional dimension.

66. The method of claim 1, wherein in the flowing step, the first fluid channel is provided in a solid substrate, the solid substrate including at least a second channel which intersects and is in fluid communication with the first fluid channel.

67. The method of claim 66, wherein a test compound is introduced into the first fluid channel through the second channel, the function of the cells being determined in the presence of the test compound.

68. The method of claim 66, wherein the solid substrate comprises a planar structure.

69. An apparatus for measuring a function of cells, comprising:

a body structure having a first channel disposed therein, the first channel being in fluid communication with a first source of a suspension of cells, wherein the cells have a first detectable property associated therewith, and wherein the cells produce a second detectable property upon activation of the function of the cells, the first and second detectable properties being distinguishable from each other;

a fluid transport system for flowing the suspension of cells along the first channel;

a detector for detecting and distinguishing the first detectable property from the second detectable property associated with cells within the first channel.

70. The apparatus of claim 69, wherein the body structure is substantially planar.

71. The apparatus of claim 69, wherein the body structure comprises at least first and second substrate layers mated together, the first channel being defined at an interface of the first and second layers.

72. The apparatus of claim 71, wherein the first channel comprises at least a first groove fabricated into a surface of at least one of the first and second layers, the groove defining the first channel when the first and second substrate layers are mated together.

73. The apparatus of claim 69, wherein the first channel comprises a cross sectional dimension between about 0.1 and 500 μm.

74. The apparatus of claim 73, wherein the first channel comprises a cross sectional dimension between about 1 and 100 μm.

75. The apparatus of claim 69, wherein the first source of suspension of cells comprises a reservoir disposed in the body structure, the reservoir being in fluid communication with the first channel.

76. The apparatus of claim 75, wherein the body structure comprises:

at least first and second substrate layers mated together, the first channel being defined at an interface of the first and second substrates; and

wherein the reservoir comprises an opening disposed through at least one of the first and second substrate layers, the reservoir being disposed in fluid communication with the first channel when the first and second substrates are mated together.

77. The apparatus of claim 69, wherein the first source of suspension of cells comprises a suspension of cells at a concentration of from about 1×10^5 to about 1×10^7 cells/ml.

78. The apparatus of claim 69, further comprising at least a second channel disposed in the body structure, the second channel being in fluid communication with the first source of the suspension of cells.

79. The apparatus of claim 69, further comprising at least a second channel disposed in the body structure, the second channel being in fluid communication with a second source of a suspension of cells.

80. The apparatus of claim 69, further comprising at least a second channel disposed in the body structure, the second channel fluidly connecting the first channel with a source of at least a first test compound.

81. The apparatus of claim 69, further comprising a source of at least a first test compound in fluid communication with the first channel.

82. The apparatus of claim 81, wherein the source of at least a first test compound comprises:

a source of a plurality of discrete test compounds; and

a test compound accessing system for separately accessing each of the discrete test compounds in the source of a plurality of test compounds, and for introducing the discrete test compounds into the first channel.

83. The apparatus of claim 82, wherein the source of a plurality of discrete test compounds comprises a plurality of reservoirs having a plurality of different test compounds separately disposed therein, and wherein the test compound accessing system comprises an external sample accessing capillary insertable into each of the reservoirs, and fluidly connected to the first channel.

84. The apparatus of claim 82, wherein the external sample accessing capillary comprises an electropipettor, the electropipettor comprising:

a capillary channel having first and second ends, the capillary channel being in fluid communication with the first channel at the first end;

first and second electrodes, the first electrode being in electrical contact with a second end of the capillary channel, and the second electrode being in electrical contact with the first channel; and

an electrical power supply coupled to the first and second electrodes for creating a voltage differential along the capillary channel.

85. The apparatus of claim 69, wherein at least one of the first and second detectable properties comprises a non-native label associated with the cells.

86. The apparatus of claim 69, wherein both of the first and second detectable properties comprise first and second non-native labels associated with the cells, respectively.

87. The apparatus of claim 69, wherein the first detectable property is substantially uniformly associated with all cells in the suspension of cells.

88. The apparatus of claim 69, wherein the first detectable property is substantially uniformly associated with a distinct subset of cells in the suspension of cells.

89. The apparatus of claim 69, wherein the first detectable property comprises a label selected from a fluorophore, a chromophore, an electrochemical label and a colloidal label.

90. The apparatus of claim 69, wherein the first detectable property comprises a label associated with nucleic acids present in the cells.

91. The apparatus of claim 69, wherein the first detectable property comprises a label associated with membranes of the cells.

92. The apparatus of claim 69, wherein the second detectable property comprises a label selected from a fluorophore, a chromophore, an electrochemical label and a colloidal label.

93. The apparatus of claim 69, wherein the function of the cells to be determined comprises a transport function, and the second detectable property being indicative of activation of the transport function.

94. The apparatus of claim 93, wherein the second detectable property comprises a fluorescent intracellular indicator compound that indicates intracellular presence of a species transported into the cells by the transport function, a fluorescent signal from the fluorescent indicator increasing or decreasing with an increasing intracellular concentration of the species within the cell.

95. The apparatus of claim 94, wherein the species transported comprises an ionic species, and the intracellular indicator is an indicator of the intracellular concentration of the species.

96. The apparatus of claim 95, wherein the species is selected from Ca^{+2}, Na^{+}, K^{+}, Cl^{-}, or H^{+}.

97. The apparatus of claim 96, wherein the species comprises Ca^{+2}, and the intracellular indicator comprises 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA) or an analog thereof.

98. The apparatus of claim 96, wherein the species comprises Ca^{+2}, and the intracellular indicator is selected from Fura-2, Fluo-2, Fluo-3. Fluo-4, Indo-1, and Calcium Green-2.

99. The apparatus of claim 96, wherein the species comprises Cl^{-}, and the intracellular indicator is selected from 6-methoxy-N-(sulfopropyl)quinolinium (SPQ), N-(sulfopropyl)acridinium (SPA), N-(6-methoxyquinolyl)acetic acid, and N-(6-methoxyquinolyl)acetoethyl ester.

100. The apparatus of claim 94, wherein the transport function is a proton transport function, and the intracellular indicator comprises an intracellular pH indicator.

101. The apparatus of claim 69, wherein the function of the cells to be determined comprises cell viability, the second detectable property being indicative of the presence of non-viable cells.

102. The apparatus of claim 101, wherein the second detectable property comprises a fluorescent indicator of cell viability, the fluorescent indicator producing a greater or lesser fluorescent signal in the presence of non-viable cells.

103. The apparatus of claim 102, wherein the fluorescent indicator is selected from a fluorogenic esterase substrate, a polyfluorinated fluorescein derivative, a polar nucleic acid based dye, a dimeric or monomeric cyanine dye, an ethidium dye and a propidium dye.

104. The apparatus of claim 69, wherein the function of the cells to be determined comprises binding of a first member of a binding pair to a second member of the binding pair, the second member being associated with the cells, wherein the second detectable property is indicative of binding of the first member of the binding pair to the second member of the binding pair.

105. The apparatus of claim 104, wherein the second detectable property comprises a fluorescent signal that increases or decreases upon binding of the first member of the binding pair to the second member of the binding pair.

106. The apparatus of claim 69, wherein the function of the cells to be determined comprises an expression function, the second detectable property being indicative of activation of the expression function.

107. The apparatus of claim 69, wherein the second detectable property comprises a fluorescent or chemiluminescent indicator that is increased or decreased upon implementation of the expression function.

108. The apparatus of claim 69, wherein the means for transporting the suspension of cells along the first channel comprises a pressure or vacuum source coupled to at least one terminus/end of the first channel

109. The apparatus of claim 108, wherein the source of the suspension of cells comprises a reservoir disposed in fluid communication with the first channel, and the pressure or vacuum source comprises a sufficient volume of the suspension of cells disposed in the reservoir to create a hydrostatic pressure differential along the first channel sufficient to flow the suspension of cells along the first channel.

110. The apparatus of claim 108, wherein the pressure or vacuum source comprises a pneumatic pressure source coupled to the source of the suspension of cells, for applying an elevated pressure to the source of the suspension of cells.

111. The apparatus of claim 108, wherein the source of the suspension of cells is in fluid communication with a first terminus of the first channel, and the pressure or vacuum source comprises a vacuum source coupled to a second terminus of the first channel.

112. The apparatus of claim 108, wherein the pressure or vacuum source comprises an electroosmotic pump coupled to the first channel.

113. The apparatus of claim 112, wherein the electroosmotic pump comprises at least a second channel disposed in fluid communication with the first channel, an interior surface of the second channel having a surface charge associated therewith, the surface charge being sufficient to support electroosmotic flow of a fluid along the second channel;

at least first and second electrodes at first and second ends of the second channel; and

an electrical power supply operably coupled to the first and second electrodes for delivering a voltage gradient therebetween.

114. The apparatus of claim 69, wherein the means for transporting the suspension of cells along the first channel comprises:

at least first and second electrodes disposed in electrical contact with different points along the first channel;

an electrical power supply coupled to the two electrodes, which power supply applies a voltage differential between the two electrodes sufficient to cause electrokinetic flow of the suspension of cells along the first channel.

115. The apparatus of claim 69, wherein the means for detecting the first and second detectable properties comprises a detector disposed adjacent to and within sensory communication of at least a first portion of the first channel.

116. The apparatus of claim 115, wherein the means for detecting detects the first and second detectable properties from a plurality of cells in the suspension of cells, simultaneously.

117. The apparatus of claim 115, wherein the first and second detectable properties comprise optically detectable properties and the detector comprises an optical detector.

118. The apparatus of claim 115, wherein at least one of the first and second detectable properties comprises a fluorescent signal, and the detector comprises a fluorescent detector.

119. The apparatus of claim 118, wherein both of the first and second detectable properties comprise fluorescent signals, and the fluorescent detector comprises an optical train for collecting fluorescent signals from the first channel and separately measuring the first detectable property from the second detectable property.

120. The apparatus of claim 69, further comprising a computer operably coupled to the means for detecting the first and second detectable properties, the computer comprising appropriate programming for comparing a level of the first detectable property to a level of the second detectable property, and determining a relative level of the function of the cells.

121. An apparatus for use in measuring a function of cells, comprising a body structure having a first fluid channel disposed therein, the first fluid channel being in fluid communication with a source of a suspension of cells, wherein the cells have a first detectable property associated therewith, and wherein the cells produce a second detectable property upon activation of the function of the cells, the first and second detectable properties being distinguishable from each other.

122. A method of measuring a binding function of a cell, comprising:

providing a channel disposed in a first body structure, the channel comprising a first binding region, the first binding region having a binding moiety immobilized on an interior surface of the first channel therein;

flowing a suspension of cells along a first channel, the cells comprising on their surfaces, a moiety specifically bound by the binding moiety;

introducing at least a first test compound into the first channel, the test compound interacting with the suspension of cells and

determining a relative velocity of cells flowing through the binding region in the presence and absence of the test compound.

123. The method of claim 122, wherein the first channel comprises at least a first non-binding region having substantially no binding moiety immobilized therein, and the step of determining the relative velocity comprises determining the velocity of cells in the non-binding region and comparing it to the velocity of cells in the binding region.

124. The method of claim 122, wherein the moiety on the surface of the cells comprises a cell surface receptor and the binding moiety comprises an adhesion molecule.

125. The method of claim 122, wherein the flowing step comprises pulsing groups of cells through the first channel.

126. The method of claim 122, wherein the flowing step comprises applying a pressure differential across the length of the first channel.

127. The method of claim 126, wherein applying the pressure differential comprises applying a hydrostatic pressure to one end of the first channel.

128. The method of claim 127, wherein applying the pressure differential comprises applying an electroosmotically driven pressure to one end of the first channel.

129. The method of claim 122, wherein the step of determining relative velocity comprises:

detecting a cell or group of cells as the cell or group of cells as the cell or group of cells passes a first point on the first channel;

detecting the cell or group of cells as the cell or group of cells passes a second point on the first channel, the first and second points being spaced apart along the first channel;

determining an amount of time for the cell or group of cells to travel along the first channel from the first point to the second point; and

calculating a velocity of the cell or group of cells from the time and a distance along the first channel between the first point and the second point.

130. The method of claim 129, wherein the cell or group of cells comprises a light emitting property, and the detecting steps comprise detecting the light emitting property.

131. The method of claim 130, wherein the light emitting property comprises fluorescence.

132. The method of claim 129, wherein the step of detecting comprises imaging the cells flowing within the first channel and measuring velocity of individual cells in an image of the first channel.

133. The method of claim 132, wherein measuring the velocity of the flowing cells in the first channel is carried out by a computer operably coupled to an imaging system which images the first channel.

134. The method of claim 122, wherein the step of introducing at least a first test compound into the first channel comprises introducing a plurality of different test compounds, serially, into the first channel.

135. The method of claim 134, further comprising the step of comparing the relative velocity of cells in the presence of the test compound with a relative velocity of cells in the absence of the test compound, an increase or decrease in the relative velocity of cells in the presence of the test compound being indicative that the test compound is an inhibitor or enhancer of cell binding to the binding moiety, respectively.

136. The method of claim 134, wherein the at least first test compounds are introduced into the main channel via an external sample accessing capillary that is fluidly connected to the first channel via at least a second channel that intersects the first channel.

137. The method of claim 136, wherein the external sample accessing capillary channel comprises an electropipettor.

138. An apparatus for measuring a binding function of a cell, comprising:

a body structure comprising at least first and second microscale channels disposed therein, the first and second microscale channels being in fluid communication at a first intersection, the first channel comprising a binding region, and a binding moiety immobilized on an interior surface of the first channel in the binding region;

a source of a suspension of cells in fluid communication with the first channel;

a means for flowing the suspension of cells along the first channel through the binding region;

a plurality of different test compound sources, each different source in selectable fluid communication with the second channel;

a means for transporting at least a first test compound from each separate test compound source through the second channel into the first channel; and

a detection system for determining a relative velocity of cells flowing through the binding region in the presence and absence of the test compound.

139. The apparatus of claim 138, wherein the source of the suspension of cells comprises at least a first reservoir disposed in the body structure, the first reservoir being in fluid communication with the first channel.

140. The apparatus of claim 138, wherein the first and second channels comprise at least one cross-sectional dimension between about 0.1 and 500μm.

141. The apparatus of claim 138, wherein the first and second channels comprise at least one cross-sectional dimension between about 0.1 and 100 μm.

142. The apparatus of claim 138, wherein the second channel is in fluid communication with a source of a plurality of different test compounds.

143. The apparatus of claim 142, wherein the second channel is in fluid communication with an external sample accessing capillary, the external sample accessing capillary being in selective fluid communication with a plurality of sources of different test compounds.

144. The apparatus of claim 143, wherein the external sample accessing capillary comprises an electropipettor in fluid communication with the second channel.

145. The apparatus of claim 138, wherein the first channel further comprises a non-binding region having substantially no binding moiety immobilized therein.

146. The apparatus of claim 138, wherein the detection system comprises at least one detector disposed in sensory communication with the first channel.

147. The apparatus of claim 138, wherein the detection system comprises at least a first and a second detector, each of the first and second detector being disposed in sensory communication with first and second points along the first channel.

148. The apparatus of claim 147, wherein the first and second detectors comprise light detectors.

149. The apparatus of claim 148, wherein the light detectors are selected from photomultiplier tubes, photodiodes and linear diode arrays.

150. The apparatus of claim 138, wherein the means for flowing a suspension of cells along the first channel comprises a source of pressure in communication with a first end of the first channel.

151. The apparatus of claim 150, wherein the source of pressure comprises a hydrodynamic pressure head in a reservoir that is fluidly connected to one end of the first channel.

152. The apparatus of claim 150, wherein the source of pressure comprises an electroosmotic pressure pump operably coupled to at least one end of the first channel.

153. The apparatus of claim 138, wherein the detection system comprises a video imaging system for recording cells flowing through the binding and nonbinding regions, the video imaging system operably coupled to a computer for determining a velocity of the cells in the binding and nonbinding regions that are recorded by the video imaging system.

154. The use of cells comprising a reference label and a function label to assay for a relative function level of the cells.

155. The use of claim 154, wherein the cells are flowed along a first channel during the assay.

156. The use of claim 154, wherein the cells are flowed along a microscale channel during the assay.

157. The use of claim 154, wherein the relative function of the cells is assayed in the presence and absence of test compounds.

158. The use of a microfluidic channel to assay for a cellular function comprising:

flowing cells along the channel, the cells comprising a reference label and a function label; and

detecting a level of reference label and a level of function label, a ratio of function label to function label providing a relative level of the cellular function.

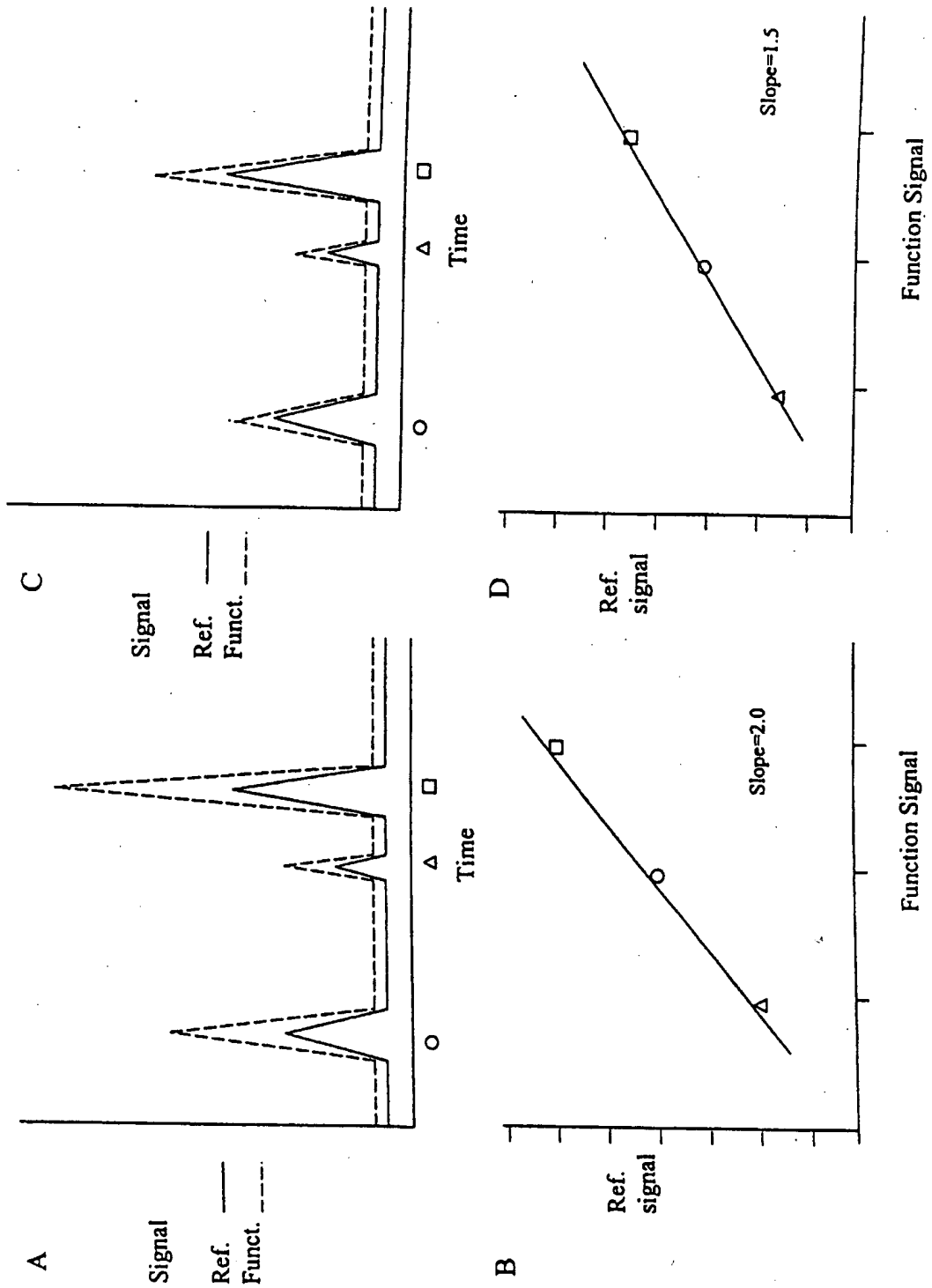
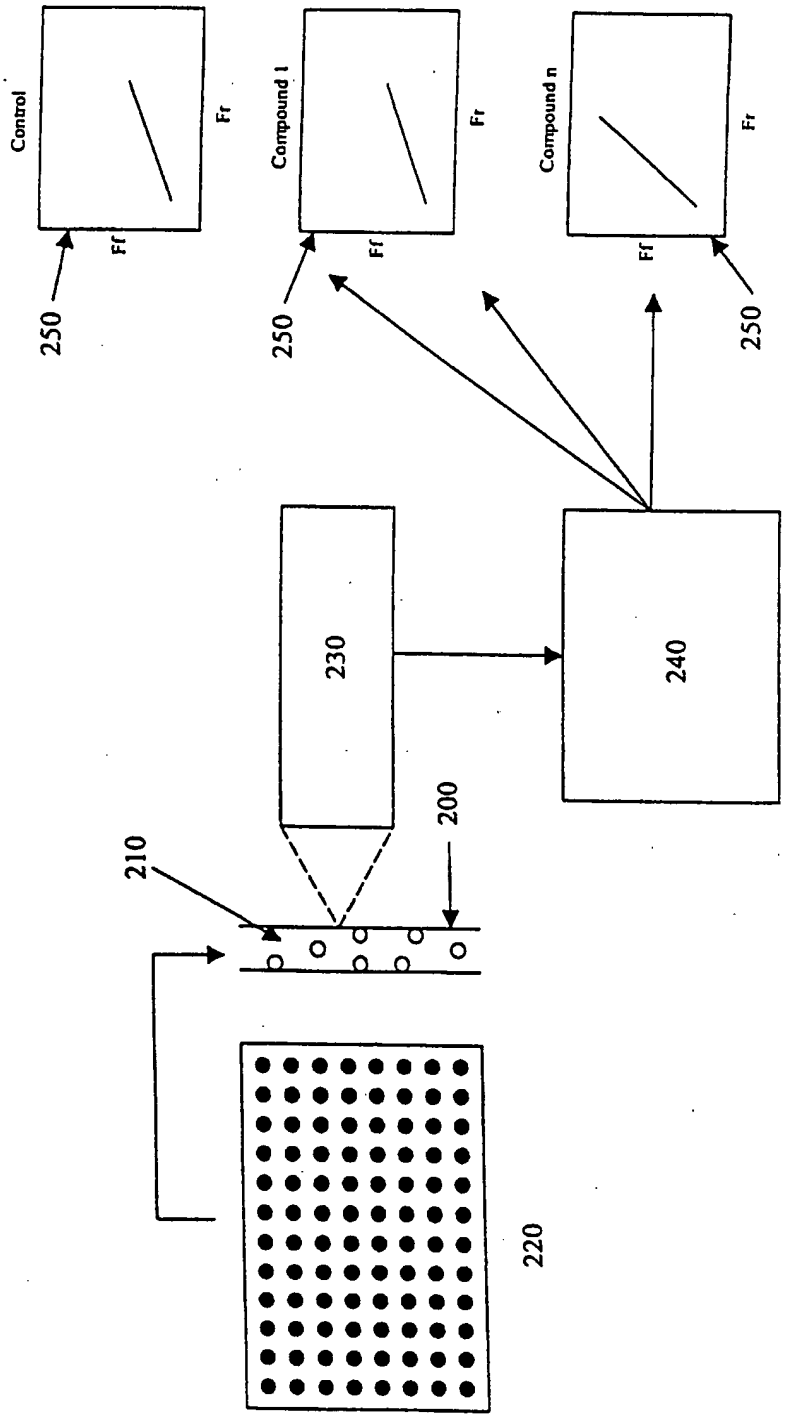

A
Signal
Ref.
Funct.
Time
B
Ref. signal
Slope=2.0
Function Signal
C
Signal
Ref.
Funct.
Time
D
Ref. signal
Slope=1.5
Function Signal

Figure 1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Ff
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Compound 1
Fr
Ff
250
Compound n
Fr
Ff
250
230
240
200
210
220

Figure 2

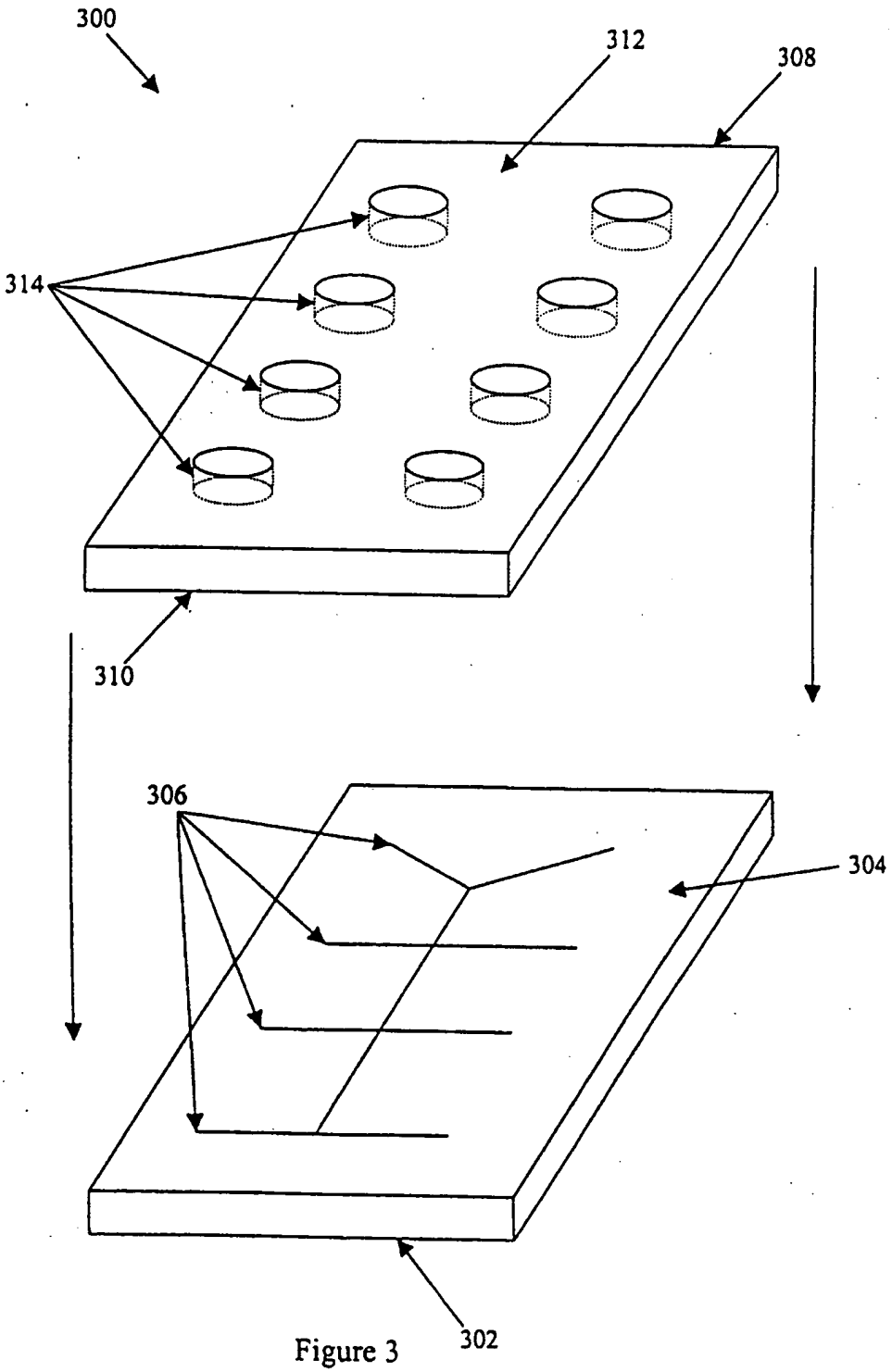

Figure 3

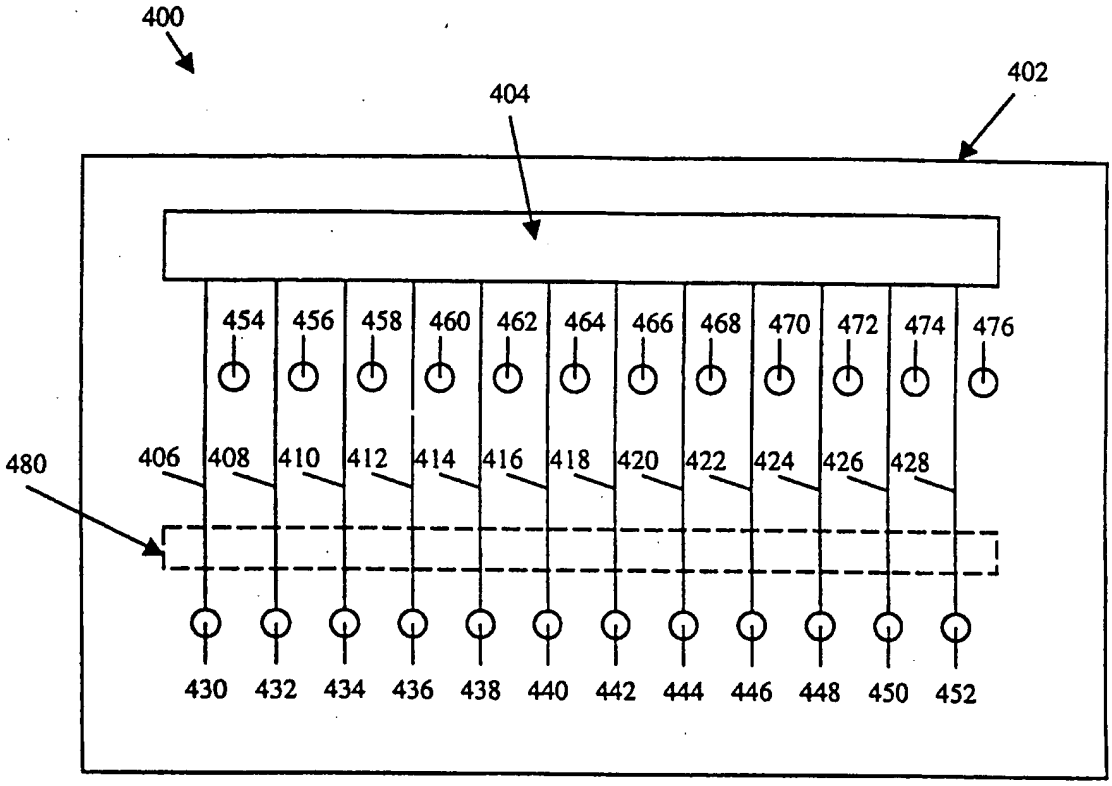
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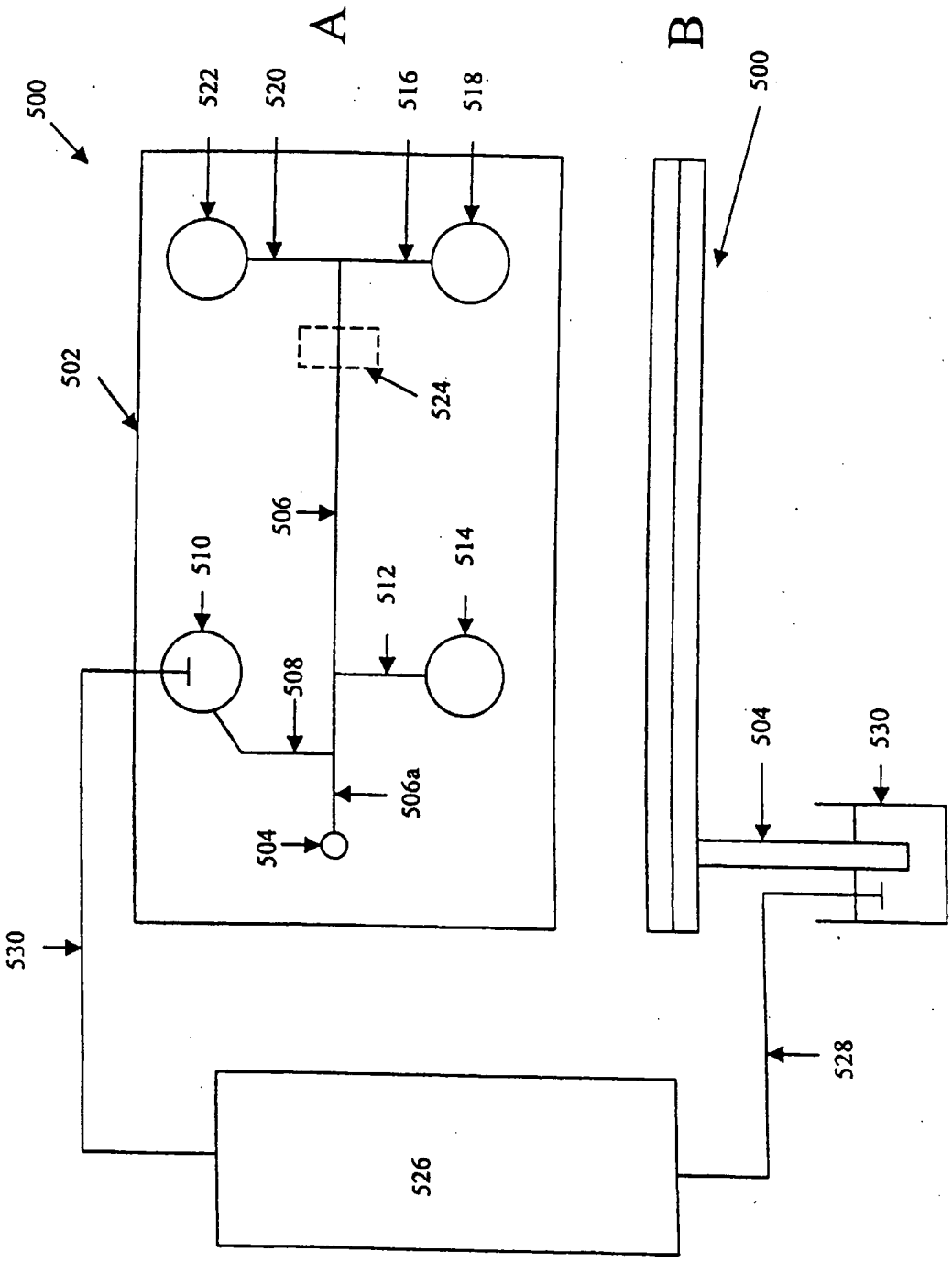
Figure 4


A
B
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512
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506a
504
530
526
528

Figure 5

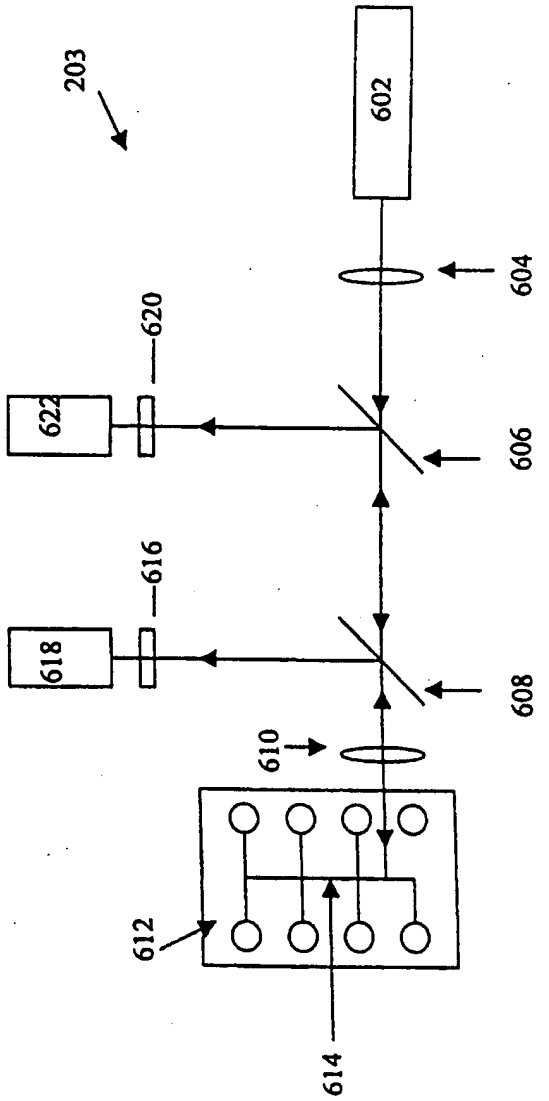

Figure 6

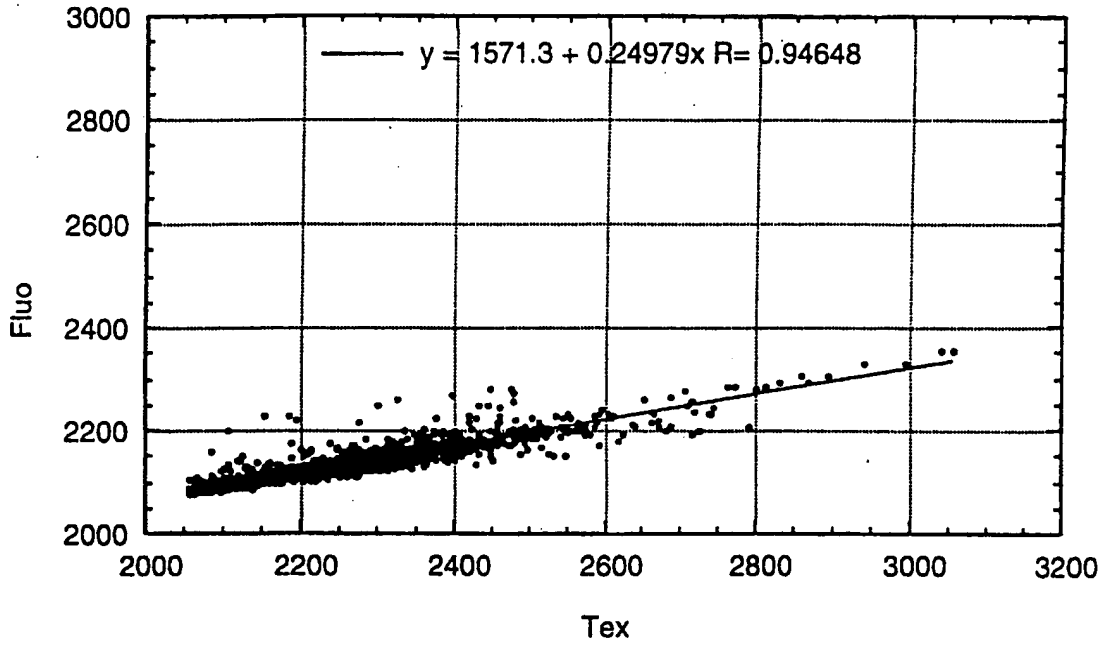

Figure 7A

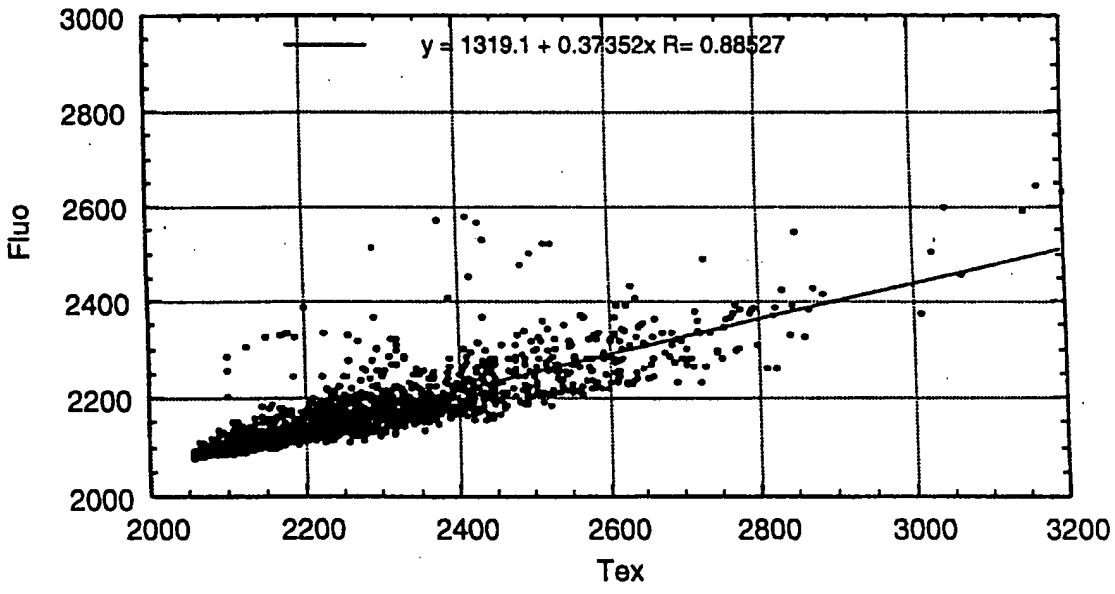

Figure 7B

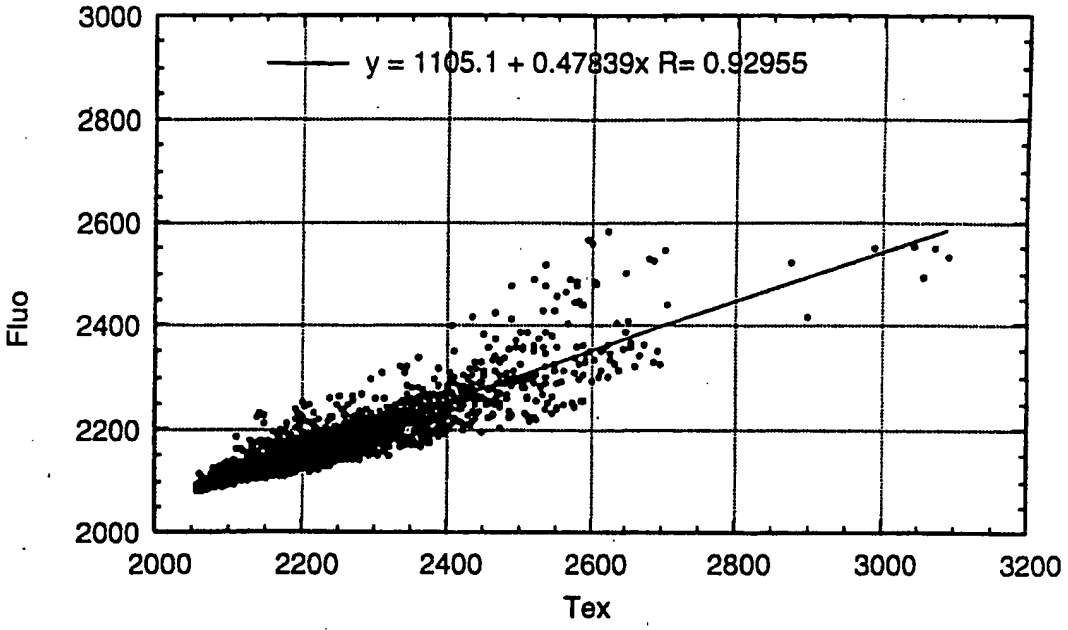

Figure 7C

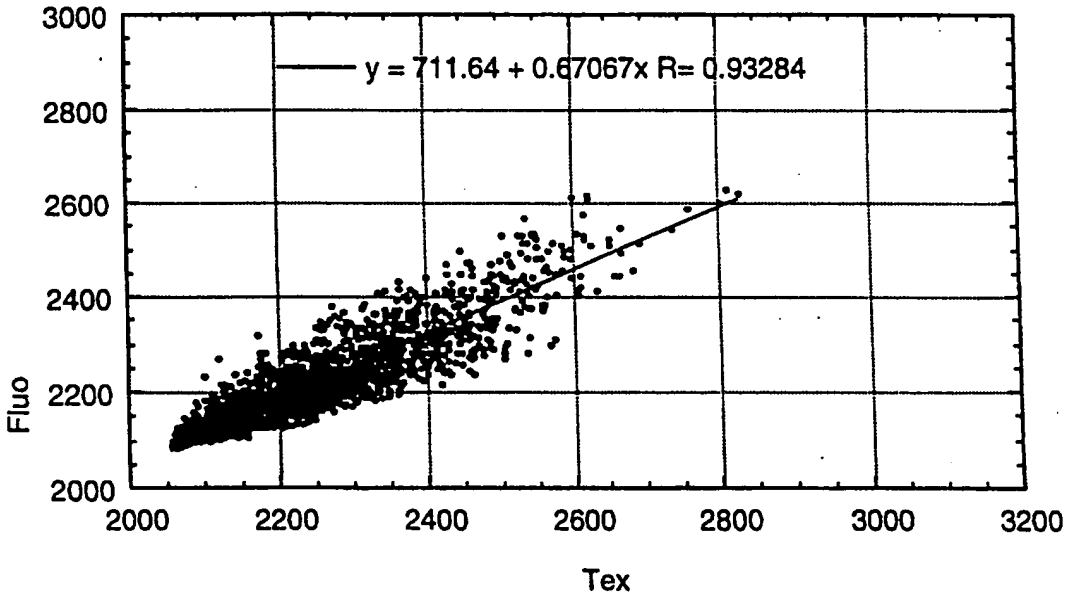

Figure 7D

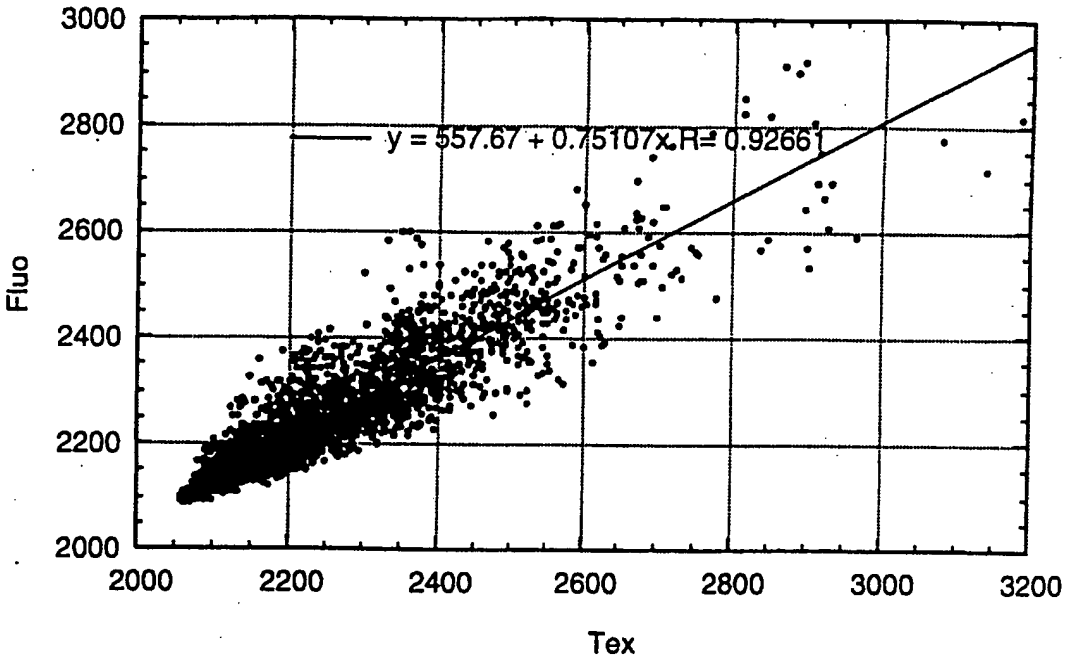

Figure 7E

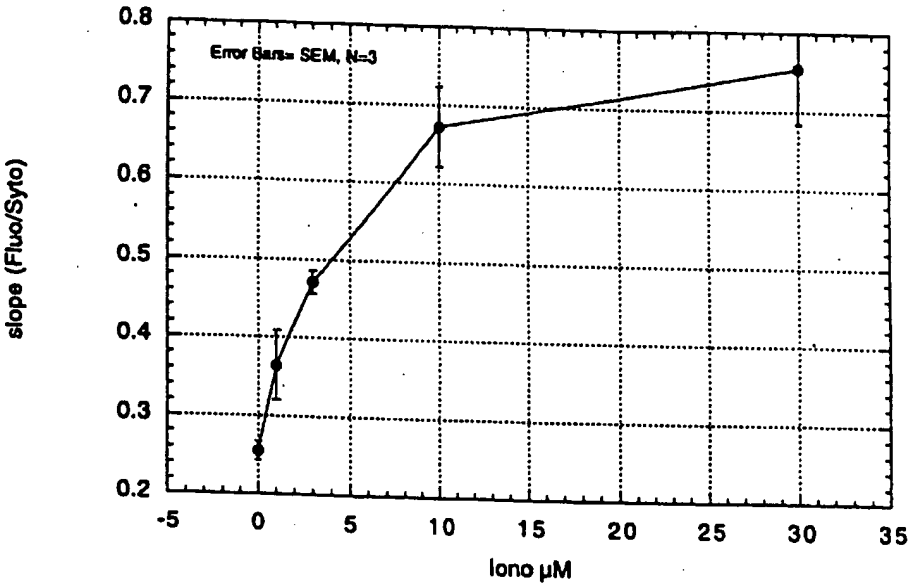

Figure 7F

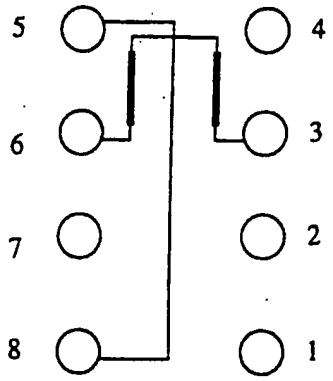

Figure 8

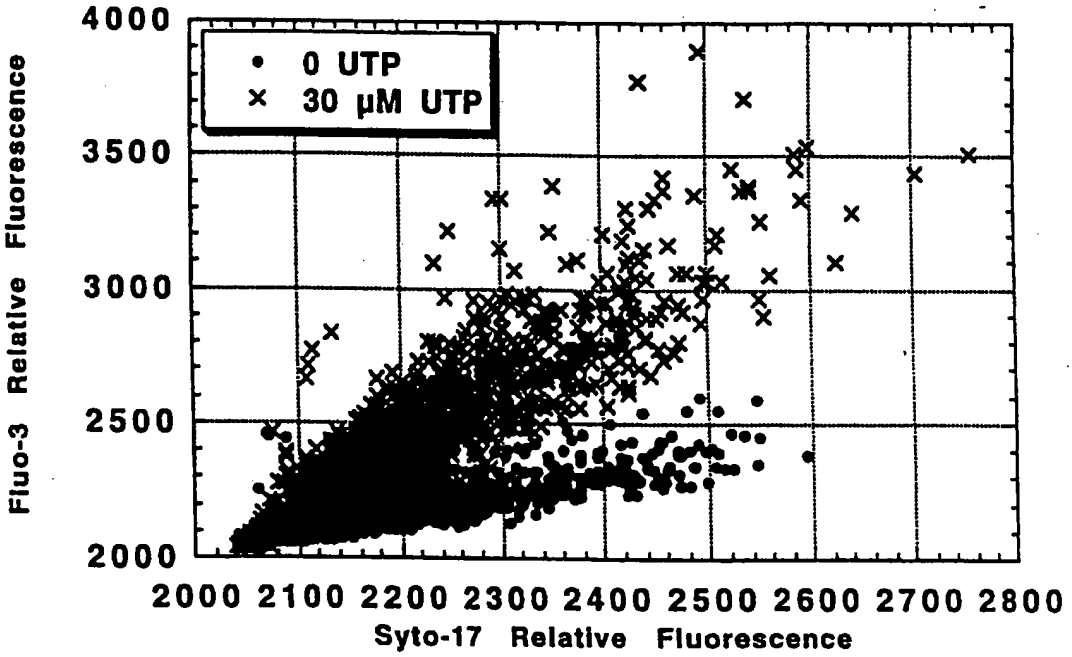

Figure 9

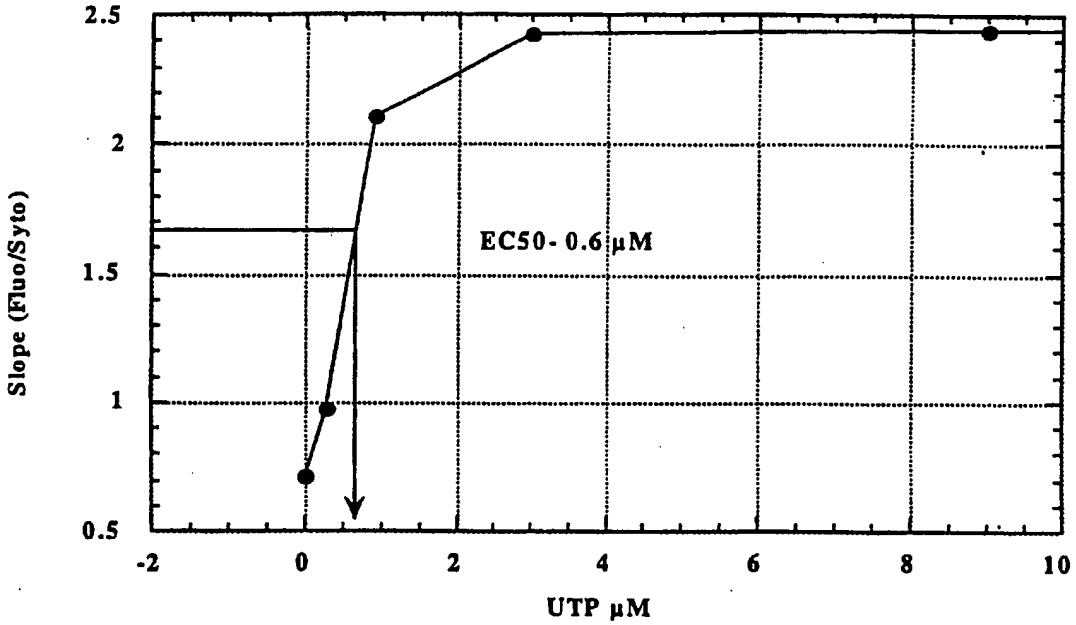

Figure 10

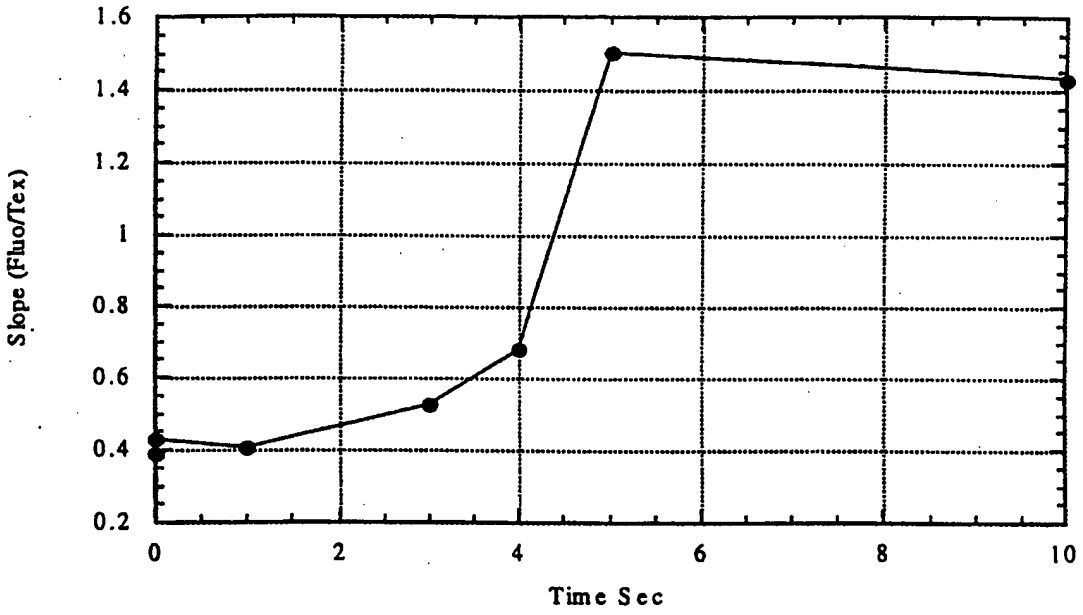

Figure 11

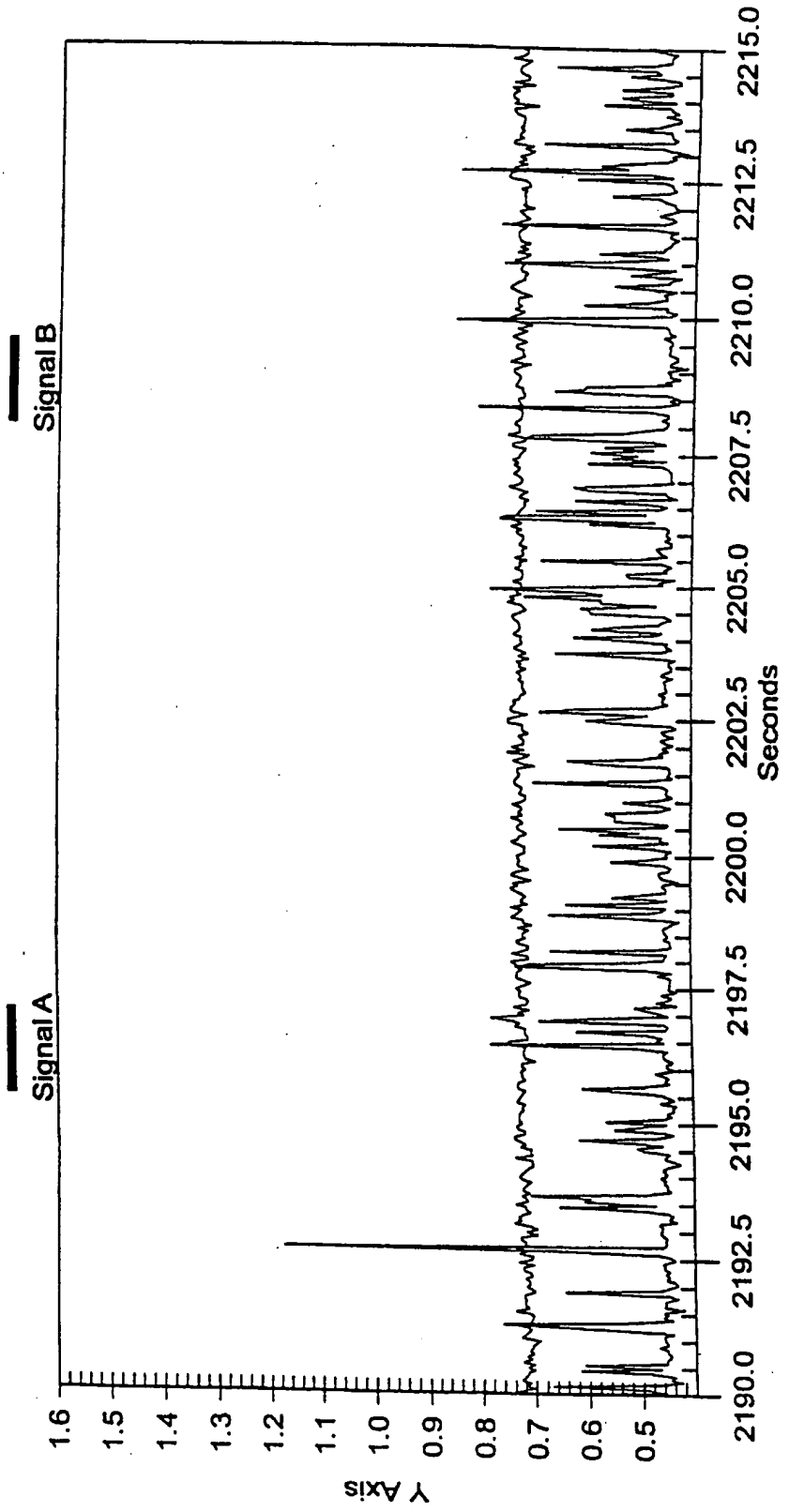
Signal B
Signal A
Y Axis
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
2190.0
2192.5
2195.0
2197.5
2200.0
2202.5
2205.0
2207.5
2210.0
2212.5
2215.0
Seconds

Figure 12

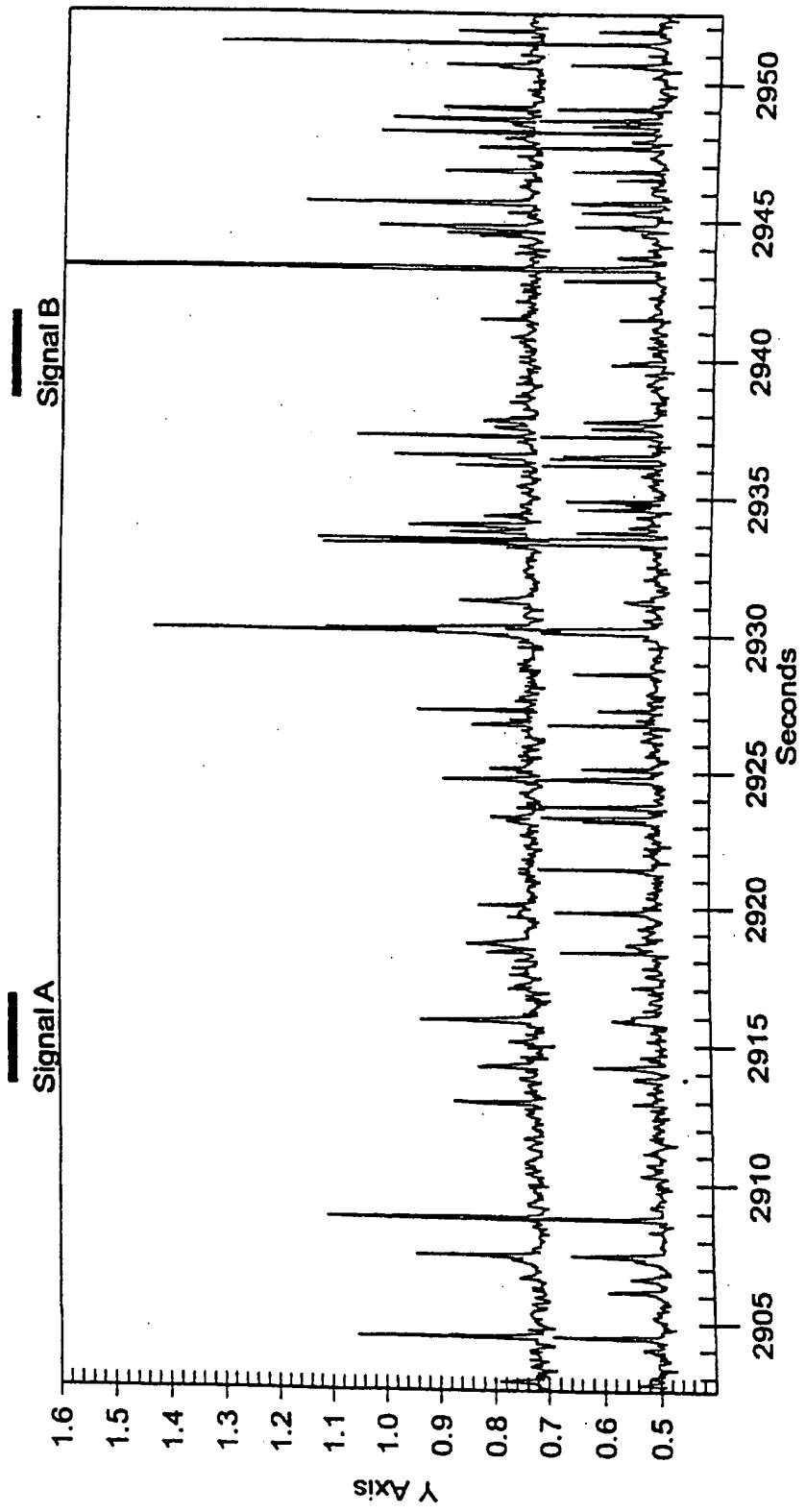
Signal B
Signal A
2905
2910
2915
2920
2925
2930
2935
2940
2945
2950
Seconds
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
Y Axis

Figure 13

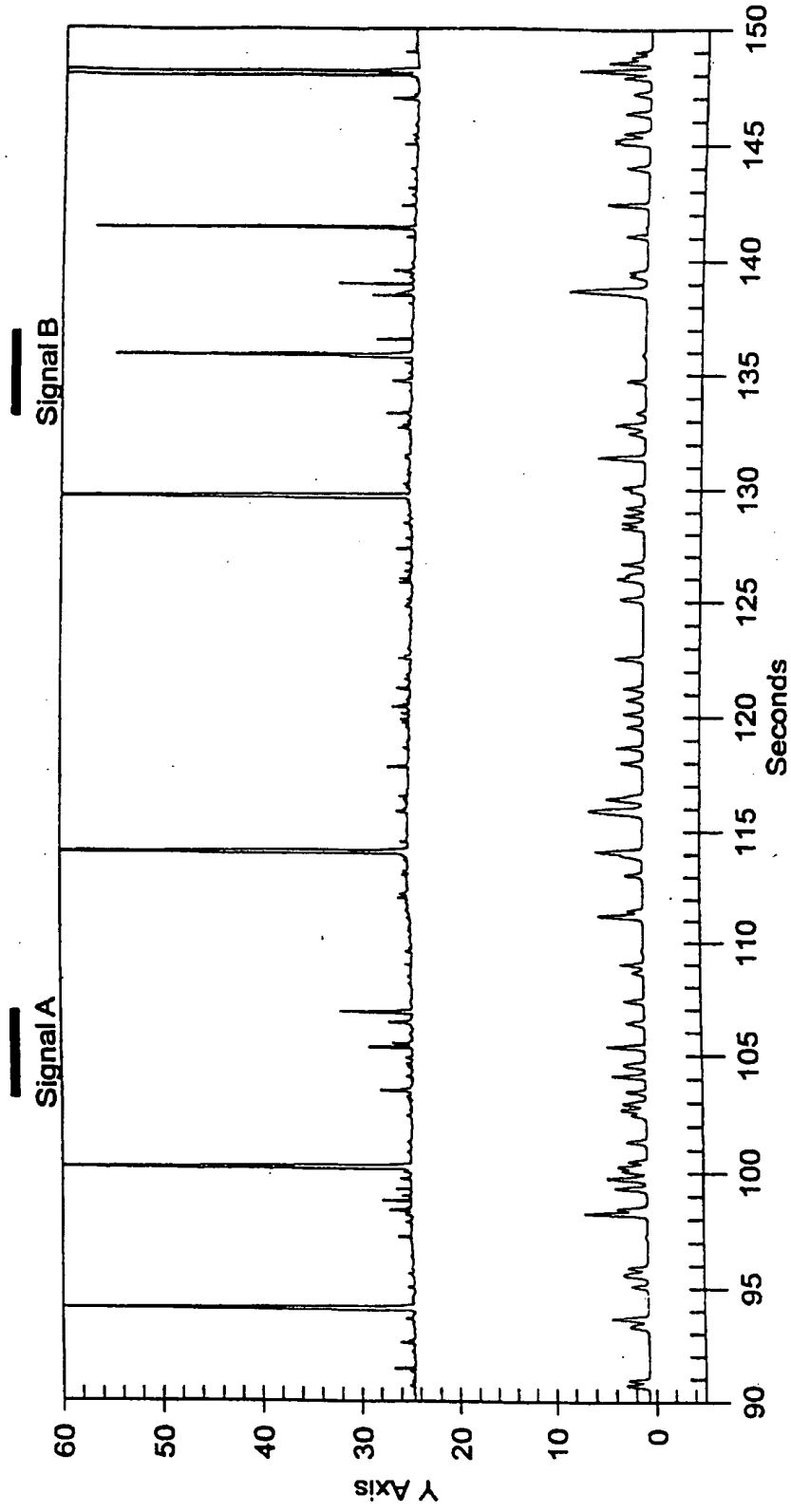
Signal A
Signal B
Y Axis
60
50
40
30
20
10
0
90
95
100
105
110
115
120
125
130
135
140
145
150
Seconds

Figure 14

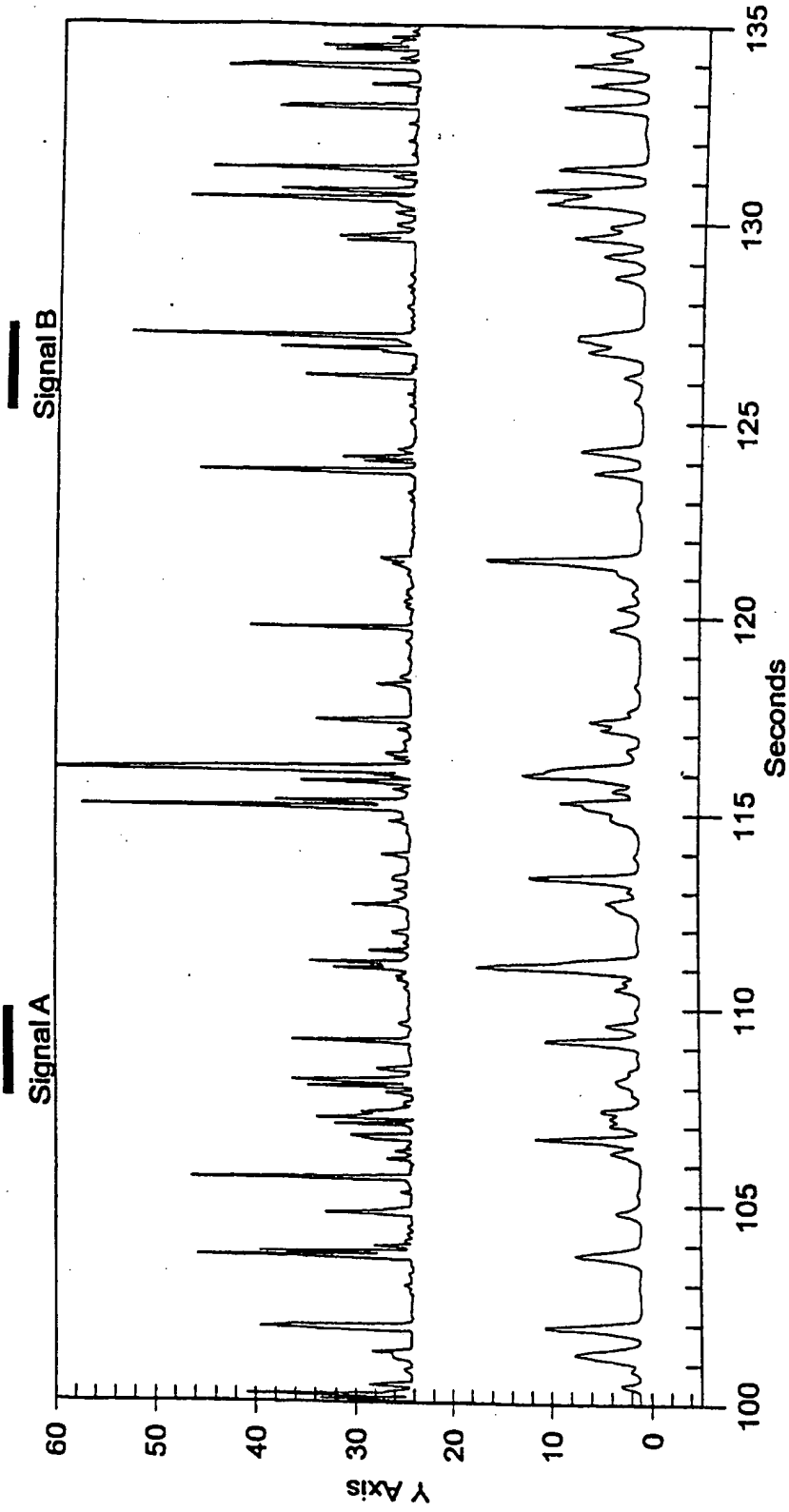
Signal A
Signal B
Y Axis
60
50
40
30
20
10
0
100
105
110
115
120
125
130
135
Seconds

Figure 15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/13918

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) : G01N 33/53; B44C 1/22
US CL : 435/7.1; 216/33
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.1; 216/33

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Please See Extra Sheet.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, P	US 5,885,470 A (PARCE et al.) 23 March 1999, see entire document.	1-158
Y	THOMAS et al. Fluorometric Assessments of Acrosomal Integrity and Viability in Cryopreserved Bovine Spermatozoa. Biology of Reproduction. 1997, Vol. 56, pages 991-998, see entire document.	1-158
Y	LAWRENCE et al. Neutrophils Roll on E-Selectin. Journal of Immunology. December 1993, Vol. 151, pages 6338-6346, see entire document.	1-158
Y	BRUNK et al. Quantifying Rolling Adhesion with a Cell-Free Assay: E-Selectin and its Carbohydrate Ligands. Biophysical Journal. June 1997, Vol. 72, pages 2820-2833, see entire document.	1-158

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
13 SEPTEMBER 1999	21 OCT 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer T.C. McCarthy III, Ph.D. Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/13918

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, P	WO 98/58074 A2 (ALLELIX BIOPHARMACEUTICALS INC.) 23 December 1998, see entire document.	1-158
Y	US 5,225,326 A (BRESSER et al.) 06 July 1993, see entire document.	1-158
Y, P	US 5,843,640 A (PATTERSON et al.) 01 December 1998. Entire document.	1-158
Y	US 5,629,147 A (ASGARI et al.) 13 May 1997, see entire document.	1-158
Y, P	US 5,891,720 A (MOORE et al.) 06 April 1999, see entire document.	1-158
Y, E	US 5,928,868 A (LIU et al.) 27 July 1999, see entire document.	1-158
Y	US 5,164,598 A (HILLMAN et al.) 17 November 1992, see entire document.	1-158
Y	US 5,637,469 A (WILDING et al.) 10 June 1997, see entire document.	1-158
Y	US 5,681,484 A (ZANZUCCHI et al.) 28 October 1997, see entire document.	1-158
Y, P	US 5,795,726 A (GLUCKSMANN) 18 August 1998, see entire document.	1-158
Y	US 5,460,945 A (SPRINGER et al.) 24 October 1995, see entire document.	1-158
Y	US 4,345,027 A (DOLBEARE) 17 August 1982, see entire document.	1-158

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/13918

B. FIELDS SEARCHED
Documentation other than minimum documentation that are included in the fields searched:

STN: HCAPLUS BIOSIS PATFULL SCISEARCH, KEYWORDS: FLUORESCENT, MICROFLUIDICS, TWO HYBRID, FLUO 3AM, SYTO 17, MULTI, ASSAY, FLOW CYTOMETRY, LIGHT